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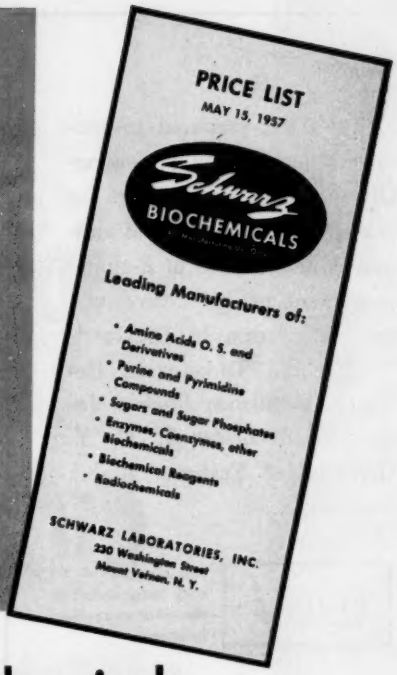

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Vol. 2, No. 7

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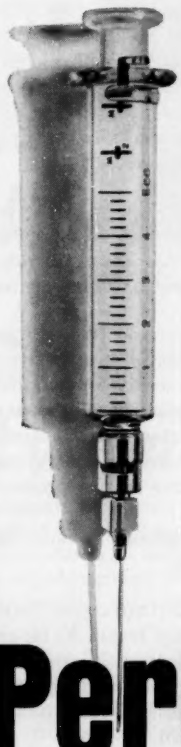

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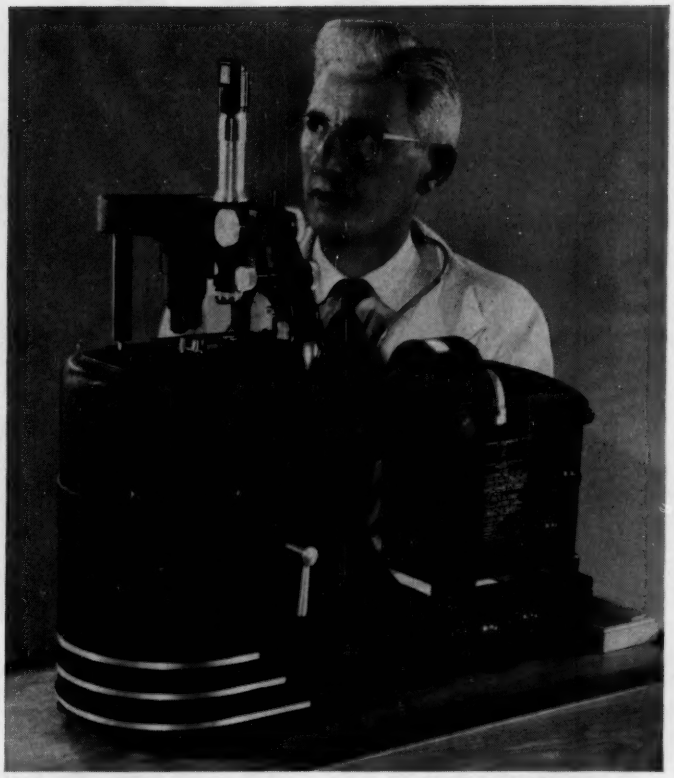

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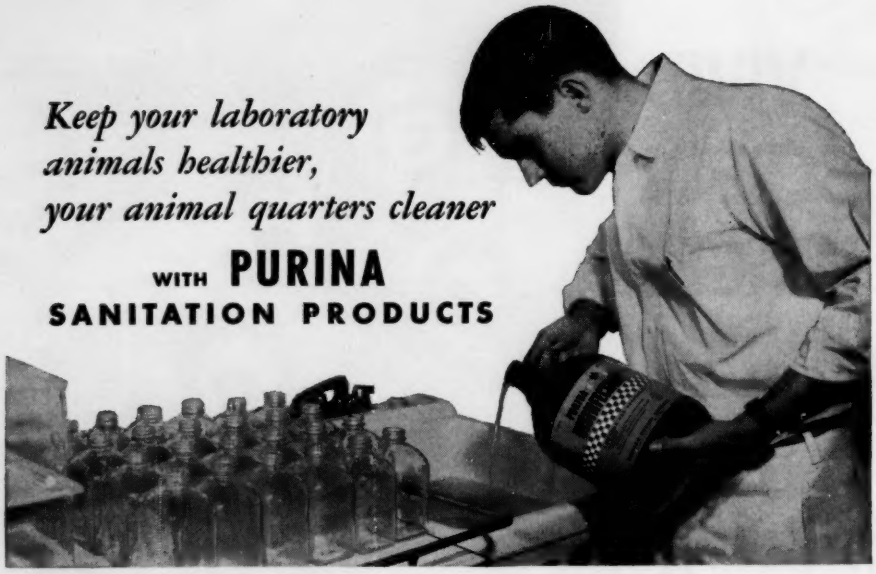

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*The American Foundation: Medical Research: A Midcentury Survey, Boston, Little, Brown and Company, 1955, vol. 1, p. XXXI.

**Ibid., p. 600.

THE HEREDITARY TRANSMISSION OF HEMOGLOBIN DIFFERENCES IN MICE [1]

By SALOME GLUECKSOHN-WAELSCH, HELEN M. RANNEY, AND BETTY F. SISKEN

(From the Department of Anatomy, Albert Einstein College of Medicine, Yeshiva University, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City, N. Y.)

(Submitted for publication December 12, 1956; accepted January 31, 1957)

The existence, in inbred strains of mice, of two different types of electrophoretic hemoglobin patterns has been demonstrated with the method of paper electrophoresis (1). Preliminary studies of the distribution of these different patterns in the inbred strains tested indicated strongly a genetic basis of hemoglobin differences in mice. It is the purpose of the present communication to report the results of breeding experiments designed to test the assumption of a hereditary transmission of these differences and to determine the mode of inheritance.

MATERIAL AND METHODS

The determination of hemoglobin patterns was carried out by filter paper electrophoresis according to the method described (2). Two types of patterns as previously illustrated (1) were obtained, one apparently of a single homogeneous protein (Type I), and the other diffuse, apparently of more than one protein (Type II). No difficulty was encountered in distinguishing pattern I from pattern II; nevertheless, minor differences between the hemoglobin patterns of individual mice within Type I or of mice within Type II groups were noted (Figure 1). Therefore, classification of hemoglobin patterns as Type I or Type II does not necessarily imply that the hemoglobin or hemoglobins present were in each case absolutely identical.

The following inbred mouse strains with hemoglobin patterns as determined in previous studies (1) and indicated below were used for breeding experiments:

Strain No.	Strain name[2]	Hemoglobin pattern
1	DBA/1	diffuse (d) Type II
2	BALB/Glw	diffuse (d) Type II
3	Fused	diffuse (d) Type II
4	C_3H	diffuse (d) Type II
5	Splotch	single (s) Type I
6	Kink I	single (s) Type I
9	Urogenital	single (s) Type I

These strains were intercrossed in a number of different combinations as follows:

Strain Nos.	Strain names[2]	Hemoglobin pattern
1 × 4	DBA/1 × C_3H	d × d
1 × 6	DBA/1 × Kink I	d × s
2 × 5	BALB × Splotch	d × s
3 × 9	Fused × urogenital	d × s
4 × 5	C_3H × Splotch	d × s
4 × 6	C_3H × Kink I	d × s
5 × 9	Splotch × urogenital	s × s
6 × 9	Kink I × urogenital	s × s

First (F_1) and second (F_2) hybrid generations as well as backcross generations were obtained and the hemoglobin patterns of the offspring tested. All animals were at least 2 months old before their hemoglobin was examined, in order to avoid any possible admixture of fetal hemoglobin.

Altogether, 635 specimens of hemoglobin were tested and distributed as follows:

F_1 generation:	140
F_2 generation:	210
Backcross of F_1 generation to parent:	102
Backcross of F_2 generation to parent:	183
	635

RESULTS

The results obtained are summarized in Tables I to IV.

As shown in Table I, crosses of strains with "diffuse" type of hemoglobin pattern with those of "single" type (experiments 1 through 5) were made up in five different combinations. Altogether, 101 offspring were tested; the hemoglobin of all of them showed the "diffuse" type of pattern.

Crosses between different strains with the "diffuse" type of hemoglobin pattern (experiment 6)

[1] Part of the work reported in this paper was carried out at the Department of Obstetrics, College of Physicians and Surgeons, Columbia University, New York. This work was supported in part by a research grant (G3676) from the National Institutes of Health, Public Health Service, and in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] The nomenclature used in the designation of mouse strains is based on the recommendations outlined by the Committee on Standardized Nomenclature for Inbred Strains of Mice (3).

TABLE I

Results of intercrosses between d and s strains—F_1 generation

Exp.	Parents	Strain numbers	Parents' hemoglobin pattern	Offspring's hemoglobin pattern	
				d	s
1	DBA × Ki	1 × 6	d × s	22	0
2	BALB × Spl	2 × 5	d × s	17	0
3	Fu × ur	3 × 9	d × s	19	0
4	C_3H × Spl	4 × 5	d × s	19	0
5	C_3H × Ki	4 × 6	d × s	24	0
	All F_1 d × s			*101*	*0*
6	DBA × C_3H	1 × 4	d × d	23	0
	All F_1 d × d			*23*	*0*
7	Spl × ur	5 × 9	s × s	0	10
8	Ki × ur	6 × 9	s × s	0	6
	All F_1 s × s			*0*	*16*

gave 23 offspring with the "diffuse" type of hemoglobin pattern.

Finally, crosses were made between different strains with "single" type hemoglobin pattern (experiments 7 and 8) and all 16 offspring tested gave the "single" pattern.

Thus "diffuse" when crossed by "diffuse" gives "diffuse," and "single" by "single" gives "single," in the first hybrid generation. "Diffuse" when crossed by "single" gives "diffuse" which by paper electrophoresis is indistinguishable from "diffuse" of the original parent with the "diffuse" type of hemoglobin pattern.

TABLE II

Intercrosses between d and s strains—F_2 generation

Exp.*	Parents' origin	Parents' hemoglobin pattern	Offspring's hemoglobin pattern	
			d	s
1	F_1 from DBA (d) × Ki (s)	d × d	26	10
2	F_1 from BALB (d) × Spl (s)	d × d	21	7
3	F_1 from Fu (d) × ur (s)	d × d	17	7
4	F_1 from C_3H (d) × Spl (s)	d × d	19	11
5	F_1 from C_3H (d) × Ki (s)	d × d	21	7
	All F_2 from d × s	*d × d*	*104*	*42*
6	F_1 from DBA (d) × C_3H (d)	d × d	26	0
	All F_2 from d × d	*d × d*	*26*	*0*
7	F_1 from Spl (s) × ur (s)	s × s	0	20
8	F_1 from Ki (s) × ur (s)	s × s	0	18
	All F_2 from s × s	*s × s*	*0*	*38*

* Numbers of experiments correspond to original F_1 crosses as listed in Table I.

Table II contains the data from further breeding tests, in which individuals of the first hybrid generation (F_1) obtained from crosses of "diffuse" by "single" were crossed *inter se* (experiments 1 through 5); 146 animals of the second hybrid generation (F_2) of all five such experiments segregated into 104 with the "diffuse" and 42 with the "single" hemoglobin pattern, a ratio approximating the expected 3:1. F_2 animals from experiment 6 (d × d) did not segregate: all the offspring gave the "diffuse" hemoglobin pattern. Absence of segregation was found also in the F_2 animals of experiments 7 and 8 where "single" was crossed by "single": all 38 offspring gave the "single" pattern.

Table III shows the results of backcrosses of F_1 animals, derived from crosses of "diffuse" by "single," to their parents with "single" hemoglobin patterns; the offspring segregated for "diffuse" and "single" with a good fit to a 1:1 ratio.

In Table IV are summarized the hemoglobin patterns of another group of backcrosses. Here, F_2 animals, from crosses of "diffuse" by "single," who showed the "diffuse" type (experiments A 1 through 5, B 1 through 5) or the "single" type (experiments C 1 through 5) of hemoglobin pattern were backcrossed to their respective grandparents with "single" hemoglobin patterns. Of the 19 F_2 animals with "diffuse" hemoglobin pattern, 7 failed to segregate and gave "diffuse" offspring only (experiments A 1 through 5). The other twelve F_2 animals (experiments B 1 through 5) when backcrossed to the grandparent with "single" hemoglobin pattern gave a segregation of "diffuse" and "single" compatible with a 1:1 ratio. The 19 animals of the second hybrid generation thus fell into 2 groups: 12 carrying the factors for both "diffuse" and "single" hemoglobin patterns, and 7 carrying the factor for "diffuse" pattern only, a segregation fitting well a 2:1 ratio of carriers to non-carriers. The F_2 animals with the "single" type hemoglobin pattern (Table IV, experiments 1 through 5), derived from the same crosses of F_1 animals as their "diffuse" siblings, were backcrossed to animals of the parent strain with "single" pattern and gave offspring with "single" hemoglobin pattern only.

In all experiments, females and males were distributed equally among the different classes of offspring.

DISCUSSION

All the results reported here fit the assumption that factors for d (diffuse) and s (single) hemoglobin patterns segregate as simple Mendelian autosomal alleles. The results of crosses of d × s (Table I) in the first hybrid (F_1), in the second hybrid generation (F_2) (Table II) as well as in the backcross generations (Tables III and IV) might be interpreted as indicating that d is dominant over s. Although the method of paper electrophoresis employed did not permit distinction of hemoglobin patterns of animals carrying the factors for both the d and the s type from the hemoglobin pattern of animals carrying the factor for d only, it is conceivable that the hemoglobin composition of d/s animals might be distinguished from that of animals homozygous for d by other methods. Further experiments are planned to elucidate this point. The decision between dominance and co-dominance of d and s can therefore not be made at present.

The distribution of hemoglobin patterns of F_2 animals and of backcrosses of both F_1 and F_2 animals to the parent strain with the "single" type hemoglobin pattern is entirely consistent with the assumption that d and s behave as alleles segregating in the expected ratios.

Comprehensive data on the genetic aspects of human hemoglobins have been summarized by Neel (4): the genetic aspects of hemoglobins in man are somewhat different from those found in mice. Human hemoglobins are controlled genetically by a group of factors some of which behave as alleles; it seems that each allele is responsible for the presence of one particular hemoglobin. With respect to human hemoglobins, then, an individual homozygous for any one of these genetic factors shows the presence of pre-

TABLE III

Results of backcrosses of F_1 generation from d × s to s strains

Exp.*	Origin of F_1 parent	BC parent	Offspring	
			d	s
2	from BALB (d) × Spl (s)	Spl (s)	25	21
3	from Fu (d) × ur (s)	ur (s)	16	23
5	from C_3H (d) × Ki (s)	Ki (s)	8	9
	All F_1 from d × s backcrossed to s		*49*	*53*

* Numbers of experiments correspond to original F_1 crosses as listed in Table I.

TABLE IV

Results of backcrosses of F_2 generation to s strains

Exp.*	Origin of F_2 parent	Hemoglobin pattern of parent	Number of animals tested	BC parent	Offspring	
A					d	s
1	F_2 from DBA (d) × Ki (s)	d	2	Ki (s)	13	0
2	F_2 from BALB (d) × Spl (s)	d	1	Spl (s)	3	0
3	F_2 from Fu (d) × ur (s)	d	3	ur (s)	17	0
5	F_2 from C_3H (d) × Ki (s)	d	1	Ki (s)	4	0
	A: *all non-segregating F_2 d*		*7 = d/d*	*s*	*37*	*0*
B						
1	F_2 from DBA (d) × Ki (s)	d	4	Ki (s)	16	13
2	F_2 from BALB (d) × Spl (s)	d	3	Spl (s)	11	9
3	F_2 from Fu (d) × ur (s)	d	1	ur (s)	11	1
4	F_2 from C_3H (d) × Spl (s)	d	1	Spl (s)	1	2
5	F_2 from C_3H (d) × Ki (s)	d	3	Ki (s)	6	8
	B: *all segregating F_2 d*		*12 = d/s*	*s*	*45*	*33*
C						
1	F_2 from DBA (d) × Ki (s)	s	6	Ki (s)	0	31
2	F_2 from BALB (d) × Spl (s)	s	2	Spl (s)	0	13
3	F_2 from Fu (d) × ur (s)	s	1	ur (s)	0	2
4	F_2 from C_3H (d) × Spl (s)	s	1	Spl (s)	0	4
5	F_2 from C_3H (d) × Ki (s)	s	5	Ki (s)	0	18
	C: *all F_2 s*		*15 = s/s*	*s*	*0*	*68*

* Numbers of experiments correspond to original F_1 crosses as listed in Table I.

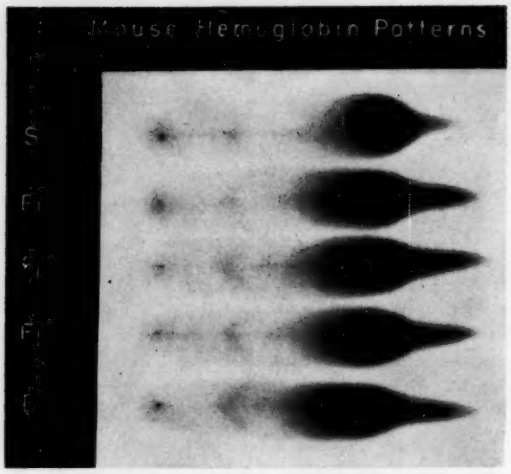

FIG. 1. PAPER ELECTROPHORETIC PATTERN OF HEMOGLOBINS OF PARENTS AND OFFSPRING

S is parent with Type I (single) hemoglobin pattern; D is parent with Type II (diffuse) hemoglobin pattern. All three F_1's are sibs and show Type II pattern.

Paper electrophoresis pattern: 6 hour run, 450 volts, stained with bromphenol blue.

dominantly one type of hemoglobin, whereas an individual heterozygous for any 2 of the allelic genetic factors will have 2 electrophoretically demonstrable different hemoglobins. However, recent studies by Kunkel and Wallenius (5), Morrison and Cook (6), and Schapira, Dreyfus, and Kruh (7) indicate that there may be more than one variety of hemoglobin in the erythrocytes, even of the normal human who is homozygous for the genetic factor for normal human hemoglobin.

In the mouse, the presence of the s allele in homozygous condition is accompanied by the presence of only one type of hemoglobin pattern, *i.e.*, the "single" type. Animals homozygous for the d allele demonstrate the "diffuse" type of hemoglobin pattern which may, however, be a mixture of hemoglobins rather than one hemoglobin. The individual heterozygous for d and s shows the same hemoglobin pattern electrophoretically as does the d homozygote. It would appear particularly interesting if a mixture of hemoglobins as is perhaps present in the d homozygote were controlled by one genetic unit; this situation would be different from that of the abnormal hemoglobins in man where each allele manifests itself by the presence of one hemoglobin.

SUMMARY

The hereditary transmission of hemoglobin differences in mice has been investigated by the method of paper electrophoresis.

Crosses between strains with two different electrophoretic hemoglobin patterns, "diffuse" (d) and "single" (s), show that the genetic factors for the 2 patterns segregate as simple Mendelian autosomal alleles.

The methods employed in this investigation do not permit differentiation of the electrophoretic hemoglobin pattern of mice of the first hybrid generation, heterozygous for "diffuse" and "single" (d/s), from the "diffuse" pattern of the parent, homozygous for "diffuse" (d/d).

The hereditary control of hemoglobin differences in the mouse appears to differ from that of abnormal hemoglobins in man.

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ALDOSTERONE EXCRETION IN NORMAL SUBJECTS DEPLETED OF SODIUM AND POTASSIUM [1]

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Aldosterone has recently been identified in adrenal cortical extracts (1) and in human urine (2, 3) as the principal steroid affecting electrolyte balance. The physiological mechanisms controlling the release of aldosterone are not completely understood (4). A number of different mechanisms and stimuli have been suggested (5–12) as important in the regulation of aldosterone secretion.

In normal subjects on diets which were normal except for decreased sodium content, aldosterone output in the urine has been previously shown to be increased (3, 13); whereas sodium loading in subjects on otherwise normal diets results in decreased output of aldosterone (14). A rise in aldosterone output has been observed during potassium loading in man (14). In rats fed a low potassium diet, the aldosterone content of adrenal venous blood was decreased (8). Human aldosterone output after potassium withdrawal from the diet has not been previously studied.

The present study was designed to determine: 1) whether low potassium intake has any effect on aldosterone output in man, 2) whether low potassium intake can prevent the increase in aldosterone output that regularly follows sodium depletion, and if so, 3) whether potassium replacement during continued sodium deprivation results in a rise in aldosterone output.

EXPERIMENTAL PROCEDURE AND METHODS

Foods of low sodium and potassium content were selected to provide the experimental diet consisting of two alternate menus of three meals per day. The diet was supplemented with a multivitamin capsule daily and with calcium caseinate as additional protein. At each meal foods were weighed, and fluids were measured to give the following composition, based on calculation from standard tables:

Menu I—2,200 calories (P 79, F 84, C 242, ethanol 20), sodium 9 mEq., potassium 9 mEq.

Menu II—2,225 calories (P 71, F 68, C 287, ethanol 20), sodium 9 mEq., potassium 9 mEq.

The diet was essentially the same as used by earlier workers (15), except for lower total calories and sodium content.

The subjects for the experiment were three men in good health, who ate the experimental diet for 26 consecutive days. In each of the three subjects the study was divided into four successive periods according to the following plan:

1. *Control* (4 days)—Sodium chloride and potassium acetate, bicarbonate and citrate were added to the diet to give a total daily intake of sodium 145 mEq. and of potassium, 85 mEq.

2. *Potassium depletion* (10 days)—Potassium supplements were omitted to give a total daily intake of sodium 145 mEq. and potassium 9 mEq.

3. *Combined depletion* (7 to 8 days)—Sodium supplement was also omitted to give a total daily intake of sodium 9 mEq. and potassium 9 mEq. On the sixth and seventh days one subject took 150 mEq. of ammonium chloride.

4. *Potassium replacement* (4 days)—Supplementary potassium was added to the basic diet to give a total daily intake of sodium 9 mEq. and potassium 99 mEq.

The supplementary sodium or potassium was taken with food and was divided as evenly as possible among the three meals. The daily potassium intake during the period of potassium replacement was slightly higher than during the control period in order not to prolong repair of the potassium deficit and because the higher dose was more convenient to measure. A measured amount of distilled water was added to the dietary fluids each day to maintain the total water intake between 2.3 and 2.7 liters, except in a few instances during the first two periods when variation in individual daily fluid intake from 1.7 to 3.4 liters was allowed.

The subjects were weighed each day, and daily total collections of urine were made. At the end of each dietary period and at intervals throughout the experiment, blood samples were drawn without stasis into syringes containing heparin. Chloride in plasma and

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TABLE I

Sodium and potassium in urine and plasma; changes in body weight

Day	Subject M					Subject J					Subject L				
	Urine			Plasma		Urine			Plasma		Urine			Plasma	
	Na mEq.	K mEq.	Body weight Kg.	Na mEq. per L.	K mEq. per L.	Na mEq.	K mEq.	Body weight Kg.	Na mEq. per L.	K mEq. per L.	Na mEq.	K mEq.	Body weight Kg.	Na mEq. per L.	K mEq. per L.
	Control				Intake	Na 145 mEq./day				K 85 mEq./day					
1			70.5					77.7					83.6		
2			70.8			142	94	77.7			131	64	83.2		
3	138	84	70.5	144	3.9	130	82	77.1	144	3.8	164	76	83.0	143	4.3
4	136	79	70.2	143	4.5	110	88	77.2	141	3.9	107	76	83.0		
	Potassium depletion				Intake	Na 145 mEq./day				K 9 mEq./day					
5	121	47	70.1			111	45	77.4			109	55	83.3		
6	122	41	70.2			87	40	76.4			80	39	82.9		
7	120	26	69.9	143	3.9	90	24	76.9	143	4.0	131	30	82.9	142	4.4
8	114	24	69.9			96	23	76.8			94	34	82.7		
9	146	20	70.0			147	18	77.0			103	21	82.9		
10	134	22	69.7	144	4.0	108	17	76.6	143	3.9	97	25	82.5	144	3.8
11	133	16	69.7			134	15	76.8			103	21	83.2		
12	128	15	69.8			143	24	76.5			170	22	82.6		
13	149	15	69.7			90	13	75.9			101	14	82.6		
14	108	13	69.9	145	4.1	122	14	76.5	143	3.4	102	23	82.9	146	3.8
	Combined depletion				Intake	Na 9 mEq./day				K 9 mEq./day					
15	74	11	69.5			98	13	76.3			118	15	82.7		
16	56	11	68.9			61	12	75.6			54	16	82.2		
17	32	12	68.8	144	3.5	35	11	75.4	143	3.7	52	12	81.6	143	3.9
18	10	13	68.6			17	12	75.2			32	12	81.6		
19	6	11	68.5	145	3.8	13	9	75.3			30	9	80.8		
20*	2	14	68.5			2	10	74.9			20	11	80.9		
21*	12	37	68.7	141	3.9	3	7	75.0	144	3.7	13	10	80.5	144	3.8
22	3	23	68.5								6	10	80.1		
	Potassium replacement				Intake	Na 9 mEq./day				K 99 mEq./day					
23	2	20	68.3			3†	16†	74.5			5	13	80.1		
24	2	45	68.5	144	4.5	5	41	74.8			10	31	80.2	142	4.7
25	11	70	68.6			17	74	75.0	145	4.2	11	53	80.6		
26	32	87	68.5	142	4.5	4	84	74.7	142	3.9	19	104	80.4	141	4.8

* One hundred and fifty mEq. of NH_4Cl added to diet.
† Potassium replacement in subject J was started on day 22.

urine was determined by the method of Schales and Schales (16), plasma CO_2 content by the Van Slyke and Neill manometric method (17), and total protein by the biuret reaction (18). Creatinine chromogens in plasma and urine were measured by the method of Bonsnes and Taussky (19), sodium and potassium by flame photometry using a lithium internal standard. Urine pH and titratable acidity were determined with the glass electrode pH meter, urine being titrated to pH 7.4; and urine ammonia was measured by the method of Van Slyke and Cullen as modified by Hawk, Oser, and Summerson (20). The 17-ketosteroids in urine were measured according to Drekter's modification (21) of the method of Holtorf and Koch; and 17-hydroxycorticosteroids by a modification of the method of Silber and Porter (22).

For estimation of aldosterone output, urine was acidified to pH 1, and a chloroform extract was made immediately. After 24 hours a second chloroform extract was made (23); this was fractionated by paper chromatography in toluene-propylene glycol. The fraction containing aldosterone was eluted and assayed in adrenalectomized rats (24). Response of the rats to the aldosterone fraction has been calculated from the observed increase in ratio of potassium to sodium output, expressed in micrograms of the desoxycorticosterone acetate (DCA) standard required for an equivalent effect. Crystalline aldosterone has averaged 36.5 times the ac-

TABLE II

Effects of ammonium chloride administration on electrolyte output in urine during sodium and potassium depletion

Day	Volume *ml./24 hrs.*	pH	Sodium *mEq./24 hrs.*	Potassium *mEq./24 hrs.*	Ammonium *mEq./24 hrs.*	Titratable acid *mEq./24 hrs.*	Chloride *mEq./24 hrs.*
			Diet: Na 9 mEq./24 hrs. K 9 mEq./24 hrs.				
18	1,770	6.29	9.9	12.7	49.5	13.3	22.3
19	1,970	6.44	6.0	11.4	48.8	9.7	16.4
			Diet: Na 9 mEq./24 hrs. K 9 mEq./24 hrs. NH_4Cl 150 mEq./24 hrs.				
20	1,555	6.26	1.7	14.1	67.2	13.0	38.9
21	2,170	5.75	12.4	36.7	108.5	23.2	113.0
			Diet: Na 9 mEq./24 hrs. K 9 mEq./24 hrs.				
22	1,935	6.18	2.7	23.2	96.5	16.2	72.6

tivity of DCA in this bioassay, and this factor was used for calculations of aldosterone output from the bioassay result.

RESULTS

In Table I the daily output of sodium and potassium in the urine is given for each of the three subjects as well as the plasma concentrations of sodium and potassium and changes in body weight.

Changes in plasma and urine electrolytes

During the period of *potassium depletion* (Table I) urine potassium losses initially exceeded potassium intake. The potassium output decreased gradually and by 10 days approached the daily intake level of 9 mEq. In all three subjects plasma potassium concentration decreased 0.4 to 0.5 mEq. per L. below the last control determination. The minimum potassium deficit during the 10-day period, calculated from dietary intake and urine loss, was of the order of 150, 145, and 195 mEq. in subjects M, J, and L, respectively.

Sodium excretion decreased initially below control levels in all three subjects, but after four days tended to return toward control values in subjects M and J. The subjects appeared to retain approximately 95, 140, and 250 mEq. of sodium, respectively, in 10 days, estimated from urinary sodium output during the control period on the same intake of sodium. Plasma sodium concentration did not change strikingly (Table I); a decrease of 1.2 to 3.6 per cent in hematocrit was observed in the three subjects.

During *combined depletion* of sodium and potassium, urinary sodium output fell below the intake of 9 mEq. by the fifth, sixth, and eighth days, respectively, in subjects M, J, and L. At this time the minimum sodium deficit, calculated from dietary intake and urine losses, was of the order of 130, 160, and 250 mEq., respectively, in subjects M, J, and L. A sharp decrease in body weight was observed early in this period, and there was a slight rise in hematocrit (4.6, 2.3, and 5.1 per cent) and in plasma proteins (0.3, 0.2, and 1.1 Gm. per cent). Potassium output in the urine decreased further. In one subject the U/P ratio for potassium reached 1.0 at a urine and plasma concentration of 3.7 mEq. per L. on the seventh day of combined depletion. Plasma potassium continued to fall in subject M and remained below control levels in all subjects (Table I). The calculated minimum deficit of potassium increased only very slightly, about 15, 2, and 25 mEq., respectively, during combined depletion of sodium and potassium. Extra-renal losses of potassium were not measured, and the period of ammonium chloride administration in subject M was not included in the above calculation.

Ammonium chloride in a dose of 150 mEq. per day was administered to subject M on the sixth and seventh days of the combined sodium and potassium depletion period. Results of urine electrolyte determinations in this subject for the last five days of the period are shown in Table II. No change in body weight occurred during the administration of ammonium chloride. If one assumes a daily loss of 2 per cent of the chloride intake in feces and sweat, there was a retention of approximately 141 mEq. of chloride. This was reflected in the increase in plasma chloride from

108 to 118 mEq. per liter. During the second day of ammonium chloride administration there was a marked increase in ammonium excretion and a slight increase in titratable acidity. Sodium excretion remained below intake levels except for an increase to 12 mEq. on the second day. Conservation of potassium was less effective, however, urinary potassium rising to 36 mEq. on the second day of ammonium chloride administration and to 23 mEq. on the day following ammonium chloride administration. The changes in chloride output are closely paralleled by changes in the sum of the cations measured.

Potassium replacement, by the addition of 90 mEq. of potassium salts to the diet, resulted in retention of potassium and a slight increase in sodium excretion. Urine potassium gradually increased and approximated dietary intake by the fourth day. The last two days of this period represent the eleventh and twelfth consecutive days of sodium deprivation; the calculated clearances of endogenous creatinine chromogens were very slightly below control levels in two subjects and 23 and 24 per cent below control levels in subject J.

During 26 days on the diet, there was a net loss of 2.0 to 3.2 Kg. in body weight of the three subjects (Table I). The small changes observed in hematocrit, potassium and sodium concentrations in whole blood, and in plasma chloride and total proteins followed no consistent pattern and are not reported in detail. During potassium depletion, plasma CO_2 combining power increased by less than 2 mM. per liter. Except for the period of ammonium chloride administration in subject M, plasma CO_2 values remained within 2.5, 3.4, and 2.7 mM. per liter of control values in the three subjects.

Changes in adrenocortical steroids

Aldosterone, 17-ketosteroids, and 17-hydroxycorticosteroids in the urine were within normal limits in all three subjects during the last two days of the control period. Observed values of these steroids throughout the experiment are plotted for each subject in Figures 1, 2, and 3. In Table III the individual aldosterone bioassay results are given with their 95 per cent confidence limits, and the 24-hour output is shown as calculated from

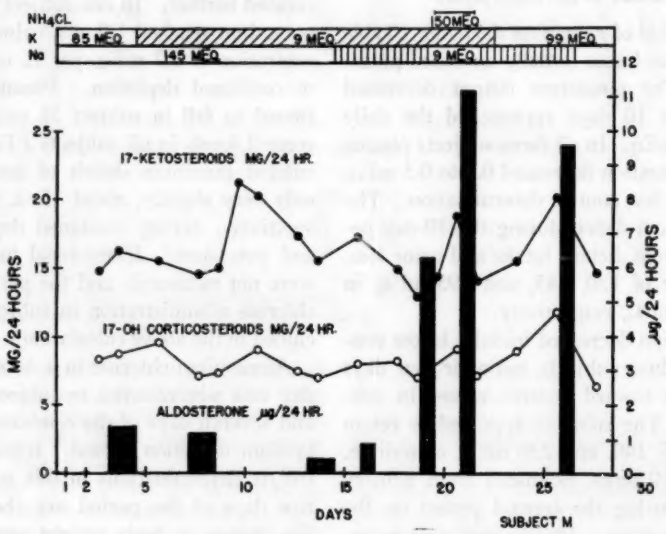

FIG. 1. STEROID OUTPUT IN URINE OF SUBJECT M

The scale for 17-ketosteroid and 17-hydroxycorticosteroid output is on the left, and for aldosterone output on the right. Dietary intake for each period is shown at the top. On the 20th and 21st days of this study, the subject took 150 mEq. NH_4Cl, during combined low sodium and low potassium intake.

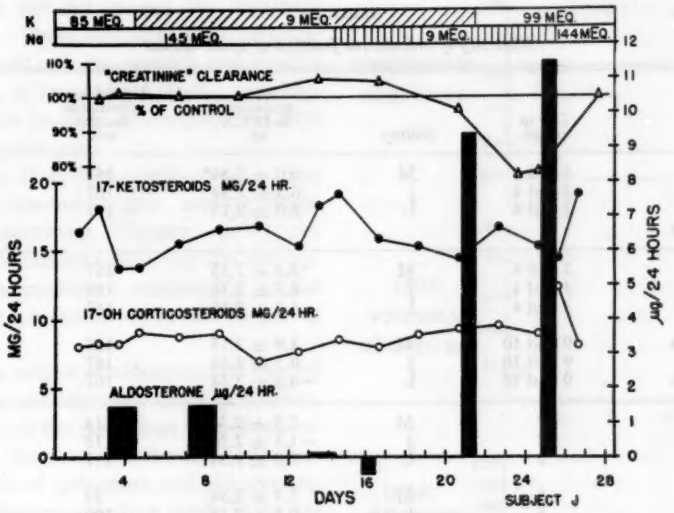

FIG. 2. STEROID OUTPUT IN URINE OF SUBJECT J

Renal clearances of endogenous creatinine are shown in this subject, in whom the changes from control values were larger than in subjects M and L.

the quantity of the aldosterone fraction of urine extract used in each assay. On the third and fourth days of potassium depletion, aldosterone output had apparently decreased almost 50 per cent in subject L but remained at normal levels in the other two subjects. On the ninth and tenth

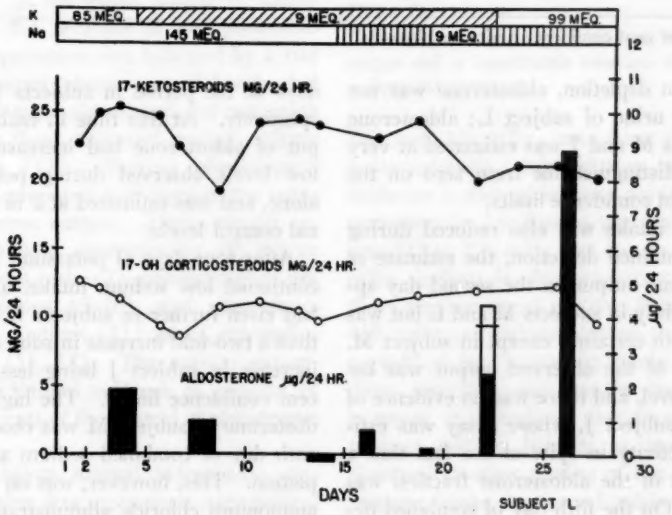

FIG. 3. STEROID OUTPUT IN SUBJECT L

Two bioassays for aldosterone output on day 22 are indicated by the solid bar and the clear bar above; the best estimate of aldosterone output on this day is shown by the horizontal line in the upper bar, this value being weighted in favor of the higher assay, which was within the dosage range for which the bioassay is standardized.

TABLE III

Bioassay of aldosterone fraction of urine extract

Period	Day in period	Subject	Effect equivalent to DOCA μg.	Dose of urine aldosterone fraction min.	Aldosterone μg./day
Control	3 and 4	M	6.0 ± 2.34*	167	1.4 ± 0.6
Normal K	3 and 4	J	6.3 ± 2.34	167	1.5 ± 0.6
and	3 and 4	L	8.0 ± 2.15	167	1.9 ± 0.5
Normal Na					
	3 and 4	M	5.2 ± 2.15	167	1.2 ± 0.5
	3 and 4	J	6.3 ± 2.34	167	1.5 ± 0.6
Low K	3 and 4	L	4.2 ± 2.15	167	1.0 ± 0.5
and					
Normal Na	9 and 10	M	1.9 ± 2.15	167	0.5 ± 0.5
	9 and 10	J	0.5 ± 2.34	167	0.1 ± 0.6
	9 and 10	L	−0.8 ± 2.34	167	0
	2	M	2.5 ± 2.34	114	0.9 ± 0.8
	2	J	−1.5 ± 2.34	115	0
	2	L	2.0 ± 2.34	117	0.7 ± 0.8
Low K					
and	5	M	5.3 ± 2.34	33	6.3 ± 2.8
Low Na	5	L	0.5 ± 2.15	100	0.2 ± 0.8
	7	J	7.9 ± 2.68	33	9.4 ± 3.2
	8	L	{2.0 ± 2.34 7.5 ± 2.15}	33 67	3.8
Low K, Low Na, and NH_4Cl	2	M	9.4 ± 2.15	33	11.2 ± 2.5
Normal K	4	M	8.0 ± 2.15	33	9.6 ± 2.5
and	4	J	9.6 ± 2.34	33	11.5 ± 2.8
Low Na	4	L	7.4 ± 2.15	33	8.9 ± 2.5

* Ninety-five per cent confidence limits for bioassay.

days of potassium depletion, aldosterone was not detectable in the urine of subject L; aldosterone output in subjects M and J was estimated at very low values, not distinguishable from zero on the basis of 95 per cent confidence limits.

When sodium intake was also reduced during the period of combined depletion, the estimate of urinary aldosterone output on the second day apparently rose slightly in subjects M and L but was not detectable with certainty except in subject M. Even in subject M the observed output was below his control level, and there was no evidence of any increase in subject J, whose assay was estimated at zero activity in spite of the fact that a maximal amount of the aldosterone fraction was used for assay. On the fifth day of combined depletion subject L continued to have urine sodium output greater than the dietary intake, and an aldosterone output that was insignificant. Sodium excretion in the urine had decreased below dietary intake on the fifth, seventh, and eighth days of the period in subjects M, J, and L, respectively. At this time in each subject the output of aldosterone had increased from the very low levels observed during potassium depletion alone, and was estimated at 2 to 6 times the original control levels.

After four days of potassium replacement, with continued low sodium intake, aldosterone output had risen further in subjects L and J, with more than a two-fold increase in subject L, the apparent increase in subject J being less than the 95 per cent confidence limits. The highest output of aldosterone in subject M was observed on the seventh day of combined sodium and potassium depletion. This, however, was on the second day of ammonium chloride administration; the effect of ammonium chloride was not studied in the other two subjects. After four days of potassium replacement, subject M appeared to have a slight decrease in aldosterone output from the previous value during ammonium chloride administration,

but this decrease did not exceed the statistical confidence limits. These estimates of aldosterone output in subject M, on the seventh and twelfth days, respectively, of low sodium intake, were significantly above the increase observed on the fifth day of sodium deprivation.

The output of 17-hydroxycorticosteroids and 17-ketosteroids remained within normal limits throughout the experiment (Figures 1 to 3); the relatively small fluctuations observed did not appear to have any consistent relationship to the changes in electrolyte intake or to the output of aldosterone.

In Figure 4 the output of aldosterone is plotted against the urinary excretion of sodium, potassium, and the logarithm of the potassium to sodium ratio in the urine. No clear relationship is evident between the output of potassium and aldosterone, but increasing aldosterone output tends to be associated with decreasing urinary sodium. A more direct relationship is apparent between the potassium to sodium ratio in urine and aldosterone output.

DISCUSSION

These studies demonstrate that low potassium intake can result in decreased output of aldosterone. Even in the presence of low potassium intake, sodium deprivation was followed by a rise in aldosterone output above control levels, but this rise occurred more slowly and did not reach as high levels as those observed during sodium deprivation alone (13). Various physiological stimuli have been proposed as factors in the regulation of aldosterone output. These stimuli include serum potassium concentration (5, 6) or potassium to sodium ratio (7), corticotrophin under certain conditions (8–10), and some function of total body sodium (11) or of extracellular fluid volume (11, 12). The present experiment provides additional evidence in support of some current concepts of the control of aldosterone output.

The decrease below normal aldosterone output observed during low potassium and normal sodium intake apparently occurred without large net losses of body potassium. The estimated deficit of potassium ranged from about 145 to 195 mEq. in the three subjects, not including losses in feces and sweat. Negative potassium balances of

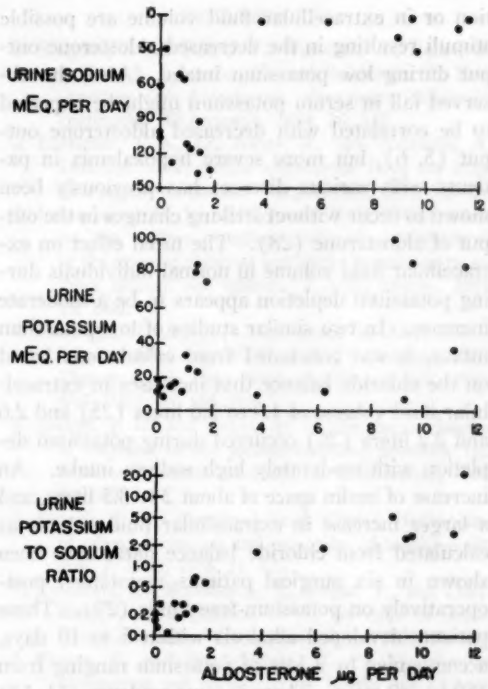

FIG. 4. ALDOSTERONE OUTPUT IN RELATION TO THE CONTENT OF SODIUM AND POTASSIUM IN THE SAME URINE COLLECTION

A direct relationship is shown between aldosterone output and a logarithmic function of the potassium to sodium output ratio.

267 and 226 mEq. have been reported (25) in a normal male subject during two seven-day periods on a diet which was similar, but lower in potassium by 7 to 8 mEq. per day. Sodium intake was 173 to 176 mEq. per day as compared to 145 mEq. sodium in the present study. These authors further noted positive balances of 569 and 417 mEq. of sodium during the two seven-day periods of low potassium intake. Estimated sodium retention was less in the present study; however, it is probable that there was significant positive sodium balance as has been regularly observed in other studies of the effect of low potassium intake in normal subjects (15, 26, 27). The diminished sodium output in the urine on the third and fourth days of the low potassium period in the present study occurred without an increase in aldosterone output.

Changes either in serum potassium concentra-

tion or in extracellular fluid volume are possible stimuli resulting in the decreased aldosterone output during low potassium intake. Also, the observed fall in serum potassium might be expected to be correlated with decreased aldosterone output (5, 6), but more severe hypokalemia in patients with various diseases has previously been shown to occur without striking changes in the output of aldosterone (28). The usual effect on extracellular fluid volume in normal individuals during potassium depletion appears to be a moderate increase. In two similar studies of low potassium intake, it was concluded from calculations based on the chloride balance that increases in extracellular fluid volume of 1.9 to 3.0 liters (25) and 2.6 and 2.2 liters (26) occurred during potassium depletion with moderately high sodium intake. An increase of inulin space of about 2 to 3.5 liters, and a larger increase in extracellular fluid volume as calculated from chloride balance data, have been shown in six surgical patients maintained postoperatively on potassium-free fluids (29). These patients developed alkalosis within 5 to 19 days, accompanied by a loss of potassium ranging from 180 to 280 mEq. There is some evidence (11, 12) that acute increases in plasma or extracellular fluid volume may be accompanied by decreased aldosterone output; this would be consistent with the findings during low potassium intake in the present study.

Under the conditions of this experiment the effect of sodium deprivation on aldosterone output appeared to predominate over the opposed effect of low potassium intake. If serum potassium concentration were the primary regulator of aldosterone secretion, as has been suggested (5), the increased output of aldosterone during the period of combined depletion would be expected to follow a rise in serum potassium concentration. During this period the measured plasma potassium concentration remained low or decreased further.

Although aldosterone output increased markedly, combined sodium and potassium depletion appears to have been associated with very small net change in plasma and extracellular fluid volume or in total body sodium. Combined sodium and potassium deprivation after a period of potassium depletion in the similar studies of Womersley and Darragh (25) was accompanied by estimated decreases of 1.0 and 1.8 liters in extracellular fluid volume during four and three days, respectively. Net sodium balance was positive in both cases. In the present study, estimated sodium retention in the three subjects of about 95, 140, and 250 mEq. during potassium depletion was followed by estimated sodium loss during combined sodium and potassium deprivation of about 130, 160, and 250 mEq. Although losses via the skin and G.I. tract were not measured, these have been small in comparable studies (30–32), and it is possible that no depletion of total body sodium occurred. The observed increases in hematocrit and plasma protein concentration immediately after sodium withdrawal were no more striking than those usually seen without previous potassium depletion, and suggest that any net change in plasma volume was small.

The continued increases in aldosterone excretion after restoration of potassium to the diet may represent a direct effect of the added potassium or possibly the effect of continued sodium deprivation. The latter seems less likely, since after eight days of combined low sodium and potassium intake, subject L had only a moderately increased output of aldosterone which was followed by a two-fold increase after four days of potassium replacement with continued sodium restriction. In contrast to the effect of potassium loading in subjects on a normal diet, the increased urine losses of sodium during potassium replacement were very slight and cannot be considered to account for the increase in aldosterone.

Corticotrophin has been shown to produce transient increases in aldosterone output (4, 10). It appears unlikely that endogenous corticotrophin played any part in the marked changes in aldosterone output during the present study, since significant variations were not observed in 17-ketosteroid and 17-hydroxycorticosteroid output. Moderate alkalosis has frequently been observed in experimental human potassium depletion (25, 33). Significant changes in plasma CO_2 combining power did not occur during the present study except briefly in the subject who was given ammonium chloride. Ammonium chloride administration during depletion of both sodium and potassium resulted in small increases in urine loss of both potassium and sodium, but predominantly of potassium. Aldosterone output rose in the

absence of any significant loss of sodium or decrease in body weight. Increased aldosterone output has been described after administration of acetazoleamide for one day (34); this was interpreted as due to sodium diuresis and loss of extracellular fluid volume.

Data of the present experiment are not consistent with the hypothesis that all changes in aldosterone secretion are regulated by extracellular fluid volume or by extracellular fluid concentration of sodium or potassium. Little change in total body sodium was produced. Relatively large changes in aldosterone output were observed without striking alterations in the concentration of plasma electrolytes, but the decreases or increases in aldosterone excretion after each change in the diet were in a direction favoring homeostasis of body sodium and potassium.

SUMMARY

1. In three normal men, restriction of potassium intake to 9 mEq. per day was followed by a marked decrease in aldosterone output below control values.

2. When sodium intake was also reduced to 9 mEq., aldosterone output rose to levels two to six times the original control values. This rise appeared to be delayed and to fall short of the increases previously observed on deprivation of sodium alone.

3. Restoration of potassium to the diet while sodium restriction was maintained was followed by a slight additional increase in aldosterone excretion in one case, and a moderate increase in the other subjects. The highest aldosterone output of one subject was observed before potassium replacement when 150 mEq. ammonium chloride was given for two days.

4. Insignificant changes were observed in the output of 17-ketosteroids and 17-hydroxycorticosteroids.

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THE EFFECTS OF CORTISONE AND HYDROCORTISONE ON WATER DIURESIS AND RENAL FUNCTION IN MAN

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The deficient diuretic response to water which is characteristic of patients with adrenal insufficiency can be rapidly corrected by the administration of cortisone (1–3). The mechanism of the defective diuresis and the precise nature of the response to hormonal replacement have not been established. A number of observations, chiefly in experimental animals, have suggested that administration of various adrenocortical preparations in large "pharmacologic" doses also influences water diuresis in the normal organism (4). However, no systematic study of the latter effect has been made in man. The present report describes the effects of large doses of cortisone and hydrocortisone on water diuresis, renal solute excretion, glomerular filtration rate, and effective renal plasma flow in man. Striking augmentation of the maximal rates of urine flow observed during maintained water loading was a regular occurrence. The steroids studied appear to have a specific influence upon water reabsorption by the renal tubules, an action which can be separated from their effect on renal hemodynamics.

MATERIALS AND METHODS

The subjects were adult male patients free from cardiovascular, renal and endocrine disease. Three sets of studies concerning water diuresis were carried out.

Group I-A—Intermittent studies during prolonged intramuscular administration of cortisone. In these experiments creatinine clearance was measured, but not inulin or para-aminohippurate clearances. Urinary total solute concentration was calculated from determined cations and urea; freezing point determinations were not made.

Group I-B—Serial studies at intervals of one to three days during prolonged oral cortisone and hydrocortisone administration. Clearances of inulin and para-aminohippurate, as well as of creatinine, were determined. Urinary osmolality was measured cryoscopically.

Group II—The acute response to intravenous infusion of hydrocortisone during water diuresis. Renal clearances and urinary total solute concentrations were measured as in I-B.

Details of experimental procedure will be given together with the results in each group.

Chemical methods and calculations

Urine and serum were analyzed for sodium, potassium, chloride and creatinine by methods previously described (5). Serum creatinine was determined after adsorption on Lloyd's reagent (6). Total solute concentration in urine and serum was measured by freezing point depression using a Fiske osmometer (Advanced Instruments, Inc., Needham, Mass.). Duplicate samples checked within 2 per cent. Results are recorded for simplicity as "mOsm. per L." although they actually are determinations of osmolal concentrations, *i.e.*, milli-osmols per kilogram of serum or urine water. Glomerular filtration rate was measured with inulin employing an anthrone method (7). Serum recoveries performed with each set of analyses averaged 98.2 per cent with a range of 94.7 per cent to 105.7 per cent. No correction was applied for the recovery error. Effective renal plasma flow was measured with para-aminohippurate (8). Clearance values were not corrected for the concentration of plasma water. Free water clearance (C_{H_2O}) was calculated as the difference between urine flow (V) and osmolal clearance (C_{Osm}) as follows:

$$C_{H_2O} = V - \frac{U_{Osm}V}{P_{Osm}}$$

Only one urine sample and one blood sample were analyzed for urea and ammonia (9) in each of the serial experiments during oral administration of steroid (Group I-B); whereas in three of the four studies of the effects of hydrocortisone infusion (Group II) these constituents were measured during successive periods throughout the experiment.

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TABLE I

Intermittent observations during prolonged intramuscular administration of cortisone *

Patient, age, diagnosis, and duration of treatment	Cortisone dose†	Day of study‡	Diet	Days on diet	Weight	Urine flow	Sodium excretion	Potassium excretion	Urea excretion	Creat. clear.	Calc. total solute conc.
	mg./day				*Kg.*	*ml./min.*	*μEq./min.*	*μEq./min.*	*μmol./min.*	*ml./min.*	*mOsm./L.*
A. D. 27 yrs.		−14	Rice	3	70.3	10.0	10	72	203	139	*37*§
Neurodermatitis	200	14	Rice	3	73.1	32.1	150	69	380	175	28
32-day course	200	21	Rice	10	71.0	24.2	57	35	398	160	30
		+10	*Ad lib.*	10	74.2	17.9	294	104	217	146	*56*
A. S. 24 yrs.		− 1	Rice	3	63.2	6.7	2	99	195	118	62
Allergic rhinitis	200	19	Rice	5	66.1	15.3	12	82	278	147	*32*
33-day course	200	26	*Ad lib.*	1	71.1	24.0	149	66	322	158	*31*
		+ 6	*Ad lib.*	2	75.3	16.5	86	101	352	155	*45*
H. S. 39 yrs.		− 1	Low salt	11	58.5	17.2	18	77	259	122	32
Asthmatic bronchitis	200	12	Low salt	24	59.3	21.6	21	50	261	129	25
45-day course	200	29	*Ad lib.*	3	60.2	33.0	209	72	380	180	32
	200	33	Rice	3	57.7	27.8	122	79	176	154	24
		+17	*Ad lib.*	17	57.9	21.8	92	78	250	147	*30*
B. H. 23 yrs.		− 1	*Ad lib.*	—‖	60.5	13.3	58	75		159	
Rheumatoid arthritis	500	14	Low salt	3	59.2	24.8	87	55		146	
14-day course		+ 6	—‖	6	58.4	16.0	26	45		119	
W. H. 51 yrs.		− 1	Low salt	7	61.4	7.7	28	56		113	
Rheumatoid arthritis	500	4	Low salt	13	62.2	10.8	2	55		116	
32-day course	500	15	Low salt	22	65.5	20.6	36	63		132	
		+10	Low salt	49	65.6	19.0	229	38		98	
		+17	Low salt	56	63.3	10.9	25	83		113	
G. S. 37 yrs.		− 2	High salt	2	54.6	11.5	85	55		121	
Rheumatoid arthritis		− 1	High salt	3	54.6	13.0	126	53		109	
14-day course	500	3	High salt	6	54.6	16.0	17	61		117	
	500	9	High salt	12	59.8	21.1	29	54		139	
	500	13	High salt	16	61.6	30.2	307	77		169	
		+19	High salt	33	54.9	13.3	51	79		107	

* Values given represent the mean renal excretion rate for 90 to 180 minutes of sustained water diuresis.
† Intramuscular cortisone acetate either 125 mg. q.6.h. or 100 mg. q.12.h.
‡ (−) and (+) sign indicate pre- and post-cortisone control days, respectively.
§ Italicized values are calculated as 2(Na + K) + urea.
‖ Patient had been eating well on an unrestricted diet prior to cortisone therapy; after therapy he was offered the same diet but ate very poorly.

EXPERIMENTAL PROCEDURE AND RESULTS

Group I. Intermittent studies during prolonged intramuscular cortisone administration (Table I) [2]

Six male patients were studied at various intervals before, during, and after the administration of cortisone acetate intramuscularly in doses of 125 mg. every six hours or 100 mg. every twelve hours for 14 to 45 days. The following diets were employed:

1) Rice Diet—Containing less than 15 mEq. of sodium per day.

2) Low Salt Diet—Containing 15 to 35 mEq. of sodium per day.

3) *Ad libitum* Diet—Sodium intake approximately 130 to 200 mEq. per day.

4) High Salt Diet—Low salt diet plus 43 mEq. of sodium every six hours as a 0.45 per cent solution of NaCl; total intake 187 to 207 mEq. per day.

About one hour after a light breakfast the subjects, who remained *seated* except to void, were given an oral water load of 20 ml. per kilogram of body weight. Urine flow, electrolyte excretion, and creatinine clearance were measured during one and one-half to three hours of sustained diuresis,

[2] A preliminary report of this work appeared in the Proceedings of the 43rd Annual Meeting of the Society for Clinical Investigation, Atlantic City, New Jersey, May, 1951 (10).

with replacement of water based upon serial weighings of the subject. Urea excretion was determined in three of the six subjects. Total osmolality of the urine was not measured cryoscopically, but when urea and ammonia were determined total urinary solute was calculated as the sum: $2(Na + K + NH_4)$ + urea.[3]

The rate of urine flow during maintained water diuresis rose in each case while cortisone was being given. The flow rate on the third and fourth day of steroid administration in the two subjects tested on these days had increased by 3.0 to 3.1 ml. per minute. The increase after the eighth day ranged from 4.4 to 22.1 ml. per minute. Augmentation of flow took place irrespective of change in sodium excretion. However, in the four subjects who were studied on more than one occasion after the eighth day of cortisone administration, the magnitude of increase appeared to vary with the contemporaneous rate of sodium excretion. The changes in natriuresis could in turn be related to dietary salt intake except in G.S. This subject received the high salt diet throughout. He retained sodium and gained weight during the first ten days of steroid administration, while his maximal urine flow almost doubled. Thereafter, with cortisone intake constant, there was little further weight gain, sodium excretion rose strikingly and concomitantly a further marked rise in flow was demonstrated. Although weight gain was the rule, subject B.H., whose urine flow increased strikingly, lost 1.3 Kg. Creatinine clearance generally increased during cortisone administration but changes in urine flow and creatinine clearance were sometimes dissociated. For example, in patient A.S. urine flow fell from 24 to 16.5 ml. per minute when cortisone was discontinued, but creatinine clearance did not change. In the three subjects whose urea excretion was measured, calculated urinary osmolality during cortisone administration was lower than the control in six of seven comparisons. In one of the studies of H.S., urine solute concentration was the same as before steroid administration, but salt had been added to the diet and total solute excretion had risen from 540 to 1060 μEq. per min.

Group I-B. Serial studies at intervals of one to three days during prolonged oral cortisone and hydrocortisone administration (Tables II and III; Figures 1 and 2)

Three men, hospitalized because of chronic skin conditions, but otherwise healthy, were studied before, during and after a two-week period of oral administration of cortisone acetate or hydrocortisone. Their basic diet provided 10 mEq. of sodium and 2,000 calories, but they were allowed to eat extra portions from this diet as desired so that in some instances sodium intake might have been as much as 25 mEq. per day. The salt-poor diet was begun three days before the first experiment, and the subjects were given water loads on several occasions before the experiments were actually performed in order to accustom them to the procedure.

The schedule of individual experiments with the dose of hormone administered to each of the three subjects is given in Table II. Equally divided doses were taken at 6-hour intervals. After the high doses of hormone were discontinued, A.B. received a daily "maintenance" dose of 37.5 mg. of cortisone for four days and R.H. received 20 mg. of hydrocortisone daily for three days. Each experiment was begun about one-half hour after a light breakfast and two hours after the last dose of steroid. The subject was weighed and a venous blood sample obtained. After a suitable priming dose, an intravenous infusion of inulin and para-aminohippurate in isotonic saline was given at the rate of 0.6 ml. per min. The subject then drank 20 ml. of water per kilogram of body weight. This load was maintained after each voiding by having the subject drink an amount of water equal to the volume of urine excreted plus

[3] In 36 urine samples, obtained during other studies of water diuresis, which varied between 33 and 72 mOsm. per L. measured cryoscopically, total solute concentration was compared with the sum: $2(Na + K + NH_4)$ + urea. The calculated values were equally distributed above and below the cryoscopic values with an average difference of 2.0 mOsm. per L. It is recognized that twice the concentration of the univalent cations exceeds the osmolal concentration provided by these electrolytes owing to incomplete dissociation and to the presence of anions of valence greater than one. However, the osmolal concentration of undetermined solutes must approximate this discrepancy in magnitude to explain the good agreement between calculated and cryoscopically determined values. Similar good agreement has been obtained in dilute urine samples which do not contain inulin or para-aminohippurate.

TABLE II

Serial studies during continued oral administration of cortisone and hydrocortisone *

Patient, age, steroid, and surface area	Day	1 Steroid dose	2 Weight	3 Urine flow (V)	4 Free water clear. (C_{H_2O})	5 Osmolar clear. (C_{Osm})	6 Sodium excretion ($U_{Na}V$)	7 Potassium excretion (U_KV)	8 Inulin clear. (C_{In})	9 Creat. clear. (C_{Cr})	10 PAH clear. (C_{PAH})	11 C_{Cr}/C_{In}	12 Filtr. fract. (F.F.)	13 V/C_{In}	14 C_{Urea}/C_{In}	15 Urine tot. sol.	16 Serum total solute: Before H_2O	17 Serum total solute: After H_2O	18 Serum Na	19 Serum K
		mg./day	*Kg.*	*ml./min.*	*ml./min.*	*ml./min.*	*μEq./min.*	*μEq./min.*	*ml./min.*	*ml./min.*	*ml./min.*		*per cent*	*per cent*		*mOsm./L.*	*mOsm./L.*	*mOsm./L.*	*mEq./L.*	*mEq./L.*
B. C., 48	C1	0	68.4	10.9	8.4	2.5	51	101	114	132	607	1.16	18.8	9.6	0.70	72	281	273	135	4.9
Cpd. F	C2	0	68.6	12.9	10.4	2.5	54	72	113	132	611	1.17	18.5	11.4	0.72	50	280	269	134	4.7
S.A.—1.85 sq. M.	1	280	68.9	13.8	11.0	2.8	44	83	(131)	139	755	1.06	17.4	10.6	0.77	52	280	272	136	4.6
	2	280	68.5	15.8	12.9	2.9	70	82	126	141	671	1.12	18.8	12.4	0.77	50	284	275	136	4.3
	3	280	69.0	16.2	13.2	3.0	76	80	127	154	714	1.21	17.8	12.8	0.78	50	282	274	137	4.2
	4	280	69.2	18.0	14.8	3.2	100	82	129	154	676	1.20	19.1	14.0	0.84	49	284	274	134	4.2
	6	240	70.0	20.5	16.8	3.7	118	109	128	156	640	1.22	20.0	16.0	0.79	49	282	274	136	4.2
	8	160	70.2	21.1	17.4	3.7	141	89	136	160	690	1.18	18.7	15.5		48	284	273	134	4.6
	10	80	69.9	19.9	16.6	3.3	145	84	124	153	569	1.23	21.8	16.0	0.72	45		268	134	4.7
	13	80	69.4	18.2	14.8	3.4	112	103	131	153	660	1.17	19.8	13.9	0.73	50	277	269	136	4.6
	15	80	69.6	19.9	16.3	3.6	136	133	128	147	596	1.15	21.5	15.6	0.74	49	279	270	136	4.6
	17	0	69.0	14.7	11.7	3.0	89	124	127	144	683	1.13	18.5	11.6	0.70	55	279	267	134	4.5
	20	0	68.8	13.5	10.7	2.8	54	159	132	147	686	1.11	19.3	10.2	0.67	56	275	265	135	4.6
	22	0	68.4	13.5	10.8	2.7	68	109	129	141	716	1.09	18.0	10.5	0.70	54	279	268	136	4.8
	30	0	68.9	(12.5)	10.0	2.5	(59)	(110)	(120)	(133)	(597)	1.11	20.1	10.4	0.67	52	276	267	135	4.8
R. H., 33	C1	0	63.8	19.2	16.4	2.8	94	79	147	160	749	1.09	19.6	13.1	0.54	40	280	271	134	4.7
Cpd. F	C2	0	63.5	18.1	15.4	2.7	47	85	124	135	744	1.09	16.7	14.6	0.66	41	286	272	140	5.2
S.A.—1.73 sq. M.	2	200	63.9	21.7	18.9	2.8	81	60	127	176	836	1.38	15.2	17.1	0.68	34	281	273	139	4.4
	4	200	64.0	22.8	20.0	2.8	70	71	133	161	782	1.21	17.0	17.2	0.73	33	284	275	138	4.5
	7	200	64.2	22.5	19.3	3.2	104	86		170						40	285	(272)	(138)	(4.6)
	8	200	64.3	24.0	20.6	3.4	99	103	140	153	797	1.09	17.6	17.1	0.67	38	286	275	140	4.4
	11	200	64.2	21.1	18.4	2.7	67	109	126	156	799	1.24	15.8	16.8	0.68	34	288	280	143	4.8
	14	200	65.2	26.3	23.0	3.3	122	106	137	161	814	1.17	16.8	19.2	0.66	35	288	283	142	5.1
	17	20	64.6	(17.9)	14.8	3.1	127	81	111	150	807	1.35	13.8	16.1	0.71	45	278	270	137	4.5
	21	0	64.4	(15.9)	12.2	3.7	50	265	135	159	767	1.18	17.6	11.8	0.63	64	279	269	136	4.4
	24	0	64.9	17.3	14.1	3.2	104	240	117	147	746	1.26	15.7	14.8	0.53	51	280	275	140	4.8
A. B., 56	C1	0	70.9	13.4	10.7	2.7	47	186	121	130	513	1.07	23.5	11.1	0.59	55	278	265	132	5.4
Cpd. E	C2	0	71.1	12.8	10.0	2.8	23	186	123	166	572	1.35	21.5	10.4	0.64	59	278	263	(135)	(5.3)
S.A.—1.86 sq. M.	1	300	71.8	13.3	10.7	2.6	18	101	138	171	608	1.24	22.8	9.7	0.62	53	277	266	133	5.1
	3	300	72.0	15.0	12.6	2.4	29	92	122	173	(472)	1.42	25.8	12.3	0.74	46	283	269	136	5.1
	5	300	72.2	17.6	14.7	2.9	77	103	123	150	546	1.22	22.5	14.3	0.78	46	285	272	(133)	(5.0)
	10	300	73.1	16.6	13.3	3.3	81	140	131	155	592	1.18	22.1	12.7	0.68	53	283	269	137	5.0
	12	300	73.3	15.1	12.5	2.6	52	115	133	171	513	1.29	25.9	11.4	0.71	47	284	271	139	4.5
	15	37.5	72.2	12.1	9.6	2.5	69	73	121	150	571	1.24	21.2	10.0	0.60	56	280	271	136	4.6
	18	37.5	71.8	15.4	12.1	3.3	63	148	132	146	597	1.11	22.1	11.7	0.65	58	282	274	135	4.8
	22	0	71.7	(12.8)	9.8	(3.0)	(34)	(176)	(128)	(152)	(628)	1.19	20.3	10.0	0.64	(63)		(268)	(130)	(4.9)
	24	0	71.7	(13.7)	10.5	(3.2)	(49)	(197)	(124)	(167)	(646)	1.35	19.2	11.0	0.70	(63)	281	(271)	(135)	(4.8)

* Columns 2 and 16 represent body weight and serum osmolarity prior to administration of a water load. All other values were obtained during water diuresis. Data in columns 3, 4 and 5 are the average of 4 collection periods; columns 6, 7, 8, 9, 10 and 15 are the average of 3 collection periods, the first period being discarded in each instance. Columns 17, 18 and 19 are the average of 2 blood samples obtained during diuresis. Values in parentheses represent the mean of one less than the number of samples specified above.

30 to 50 ml. per hour for insensible loss. After the urine flow had reached a peak, four consecutive 20 to 30 minute collections were obtained by voluntary voiding.[4] Venous blood samples were obtained at the midpoint of three of the four clearance periods. The data for urine flow, osmolal clearance and free-water clearance recorded in Table II are the average of all four collection periods. Only the last three periods were averaged to obtain mean values for clearances of inulin, para-aminohippurate and excretion rates of individual solutes. The first high-flow period was discarded in order to avoid washout errors during rising urine flow. Data for the last experiment on B.C. and the last two experiments on A.B. are based on only three urine collection periods, of which the last two were averaged to provide mean values for inulin clearance, para-aminohippurate clearance and excretion rates of individual solutes. In R.H. and A.B. the water loading was performed daily throughout the period of study, regardless of whether clearances were being measured.

The maximal urine flow following a water load rose during steroid administration in each subject (Table II). In B.C. the control rates were 10.9 and 12.9 ml. per min. The rate increased progressively to 21.1 ml. per min. on the eighth day of hydrocortisone (Figure 1). In R.H. flow increased from control values of 19.2 and 18.1 to 26.3 ml. per min. on the fourteenth day, the last on which hydrocortisone was given in high doses. The oldest subject studied, A.B., had the smallest increase, from controls of 13.4 and 12.8 to a peak of 17.6 ml. per min. on the fifth day of cortisone administration.[5] There was no further rise in this subject despite continued administration of steroid. Maximal urine flow decreased soon after the steroid was discontinued, or the dose decreased to a maintenance amount. In all three subjects osmolal clearance changed only slightly, never increasing by more than 1.2 ml. per min. so that the change in urine flow was largely due to an increase in free-water clearance.

TABLE III

Correlation coefficients (r) and their significance (p) for C_{H_2O} vs. $U_{Na}V$, C_{Osm}, and weight, for data shown in Figure 2

	B. C.		R. H.		A. B.	
	r	p	r	p	r	p
$U_{Na}V$ *vs.* C_{H_2O}	.969	<.001	.896	<.02 >.01	.886	<.02 >.01
C_{Osm} *vs.* C_{H_2O}	.898	<.001	.716	<.10 >.05	−.052	>.10
Weight *vs.* C_{H_2O}	.917	<.001	.878	<.01 >.001	.596	>.10

Although glomerular filtration rate (C_{In}) was somewhat higher during hormone administration in each subject, the differences were small. When the data for all three subjects were combined, the average of control observations (including studies on a maintenance dose) was 124.7 ml. per min. (standard error ± 2.3 ml. per min.) and the combined average of all studies during steroid administration (except on maintenance dose) was 130.0 ± 1.2 ml. per min. ($p < 0.05$). The differences in filtration rate did not reach conventional levels of significance when the data for each subject were considered individually, or when studies on maintenance doses of steroid were grouped with those on higher doses. Since the rate of urine flow after water loading invariably rose during steroid administration and filtration rate usually increased the two changes were necessarily often associated. However, increase in urine flow did not parallel augmentation in C_{In}. Thus B.C. manifested a marked rise in C_{In} on the first day of steroid administration with little rise in flow and no rise in the fraction of filtered water excreted (V/C_{In}). Thereafter his flow rate rose progressively with virtually no further change in C_{In}. Moreover, after hydrocortisone was discontinued flow and V/C_{In} fell almost to the range of the control period, while C_{In} remained above control values. In R.H. the initial rise in flow and

[4] To assess the completeness of voluntary voiding the variation in creatinine output was ascertained in each subject as suggested by Mills, Thomas, and Yates (11). In 114 clearance periods of 38 studies the average deviation of creatinine excretion in any single period from the day's mean was 3 per cent. In 112 periods the deviation from the mean was no greater than 8 per cent. In the other two periods, the deviations were 10 and 13 per cent.

[5] A.B. was also the only subject who received oral cortisone. However very large increments in flow were observed in subjects receiving intramuscular cortisone (Table I).

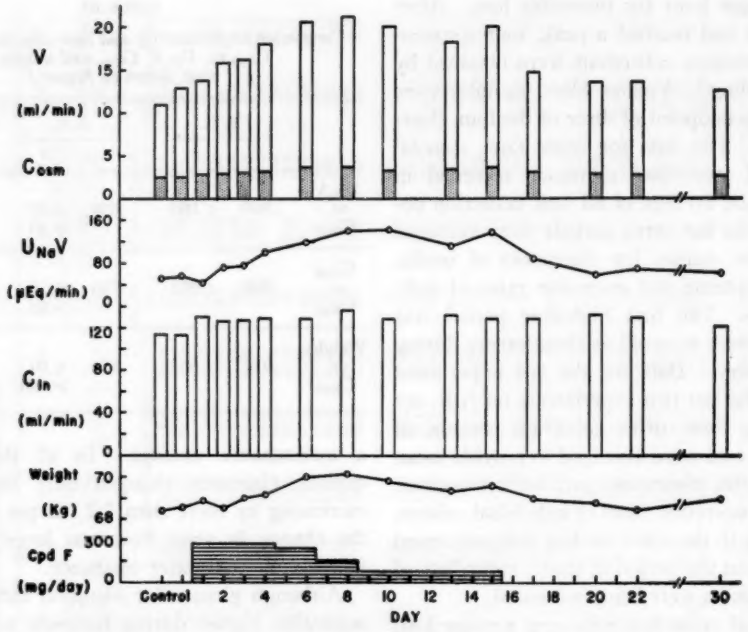

FIG. 1. SUBJECT B.C.—EFFECTS OF HYDROCORTISONE ON URINE FLOW (V), OSMOLAR CLEARANCE (C_{osm}), SODIUM EXCRETION ($U_{Na}V$), GLOMERULAR FILTRATION RATE (C_{In}) AND WEIGHT, DURING WATER DIURESIS

At the top of the figure, V is shown by bars, the lower stippled portions of which represent C_{osm}, the clear areas, free water clearance (C_{H_2O}).

V/C_{In} was unassociated with elevation of C_{In}. In A.B. the highest rate of urine flow was observed on the fifth day of hydrocortisone administration when C_{In} was at control levels.

Effective renal plasma flow (C_{PAH}) showed more random variation than C_{In}. No consistent trend was observed in subjects B.C. or A.B. In R.H., the youngest subject, C_{PAH} was consistently higher during steroid administration; during the control periods before and after steroid administration, his average renal plasma flow was 763 ± 13 ml. per min. whereas it was 806 ± 10 ml. per min. ($p < 0.05$), while hormone was being given.

Endogenous creatinine clearance generally paralleled inulin clearance, although in individual experiments the ratio of creatinine to inulin clearance (C_{Cr}/C_{In}, Table II) ranged between 1.08 and 1.40. The average C_{Cr}/C_{In} ratio was 1.21 ± 0.02 during control studies and 1.18 ± 0.02 during steroid administration.

The data concerning urea clearance, which represent one period in each experiment, are presented in terms of the ratio to inulin clearance (C_{Urea}/C_{In}, Table II). This expression minimizes errors due to bladder emptying. In each subject there was an increase in the ratio of urea to inulin clearance during steroid therapy. In subject B.C., for whom the most extensive data are available, C_{Urea}/C_{In} averaged 0.69 ± 0.01 during control studies and 0.77 ± 0.01 during steroid administration ($p < 0.01$). The serum urea concentration was not affected by steroid administration.

In addition to the increase in free-water clearance (C_{H_2O}) during steroid administration there was an increase in sodium excretion ($U_{Na}V$), osmolal clearance (C_{osm}), and the body weight. When hormone administration was discontinued, C_{H_2O} fell promptly. However, $U_{Na}V$, C_{osm}, and weight all remained elevated above pre-steroid control values in subjects R.H. and A.B.; so that there was no correlation between these variables and water excretion during the period after steroid

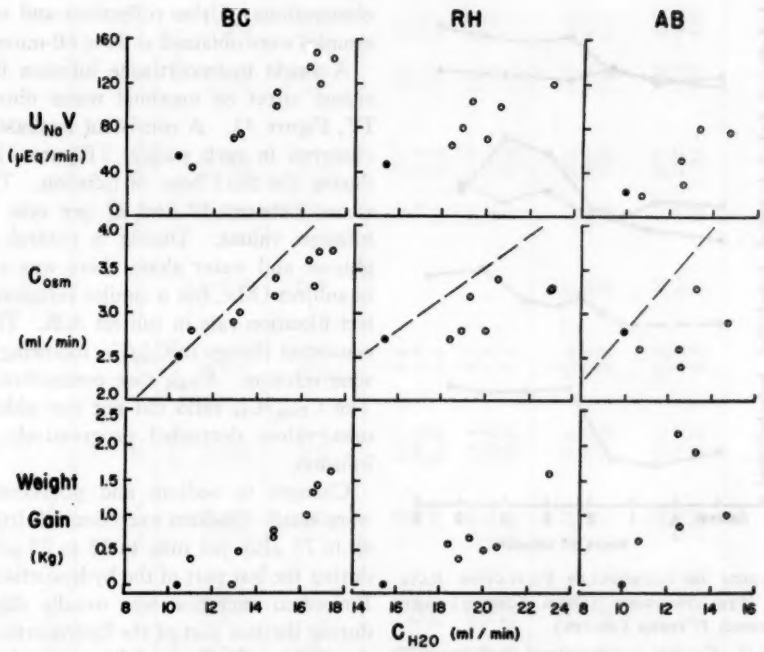

FIG. 2. THE RELATIONSHIP BETWEEN FREE WATER CLEARANCE (C_{H_2O}) AND SODIUM EXCRETION ($U_{Na}V$), OSMOLAR CLEARANCE (C_{Osm}) AND WEIGHT GAIN IS SHOWN IN SCATTER DIAGRAMS FOR SUBJECTS B.C., R.H. AND A.B.

Closed circles represent the second of two control determinations; open circles represent values obtained on high doses of steroids. The dashed lines on the charts showing C_{Osm} *vs.* C_{H_2O} indicate the relationship that would exist if urinary dilution remained at the control value. Except for one period in B.C. all points during steroid administration fall to the *right* of the line, indicating a urine more dilute than the control.

was discontinued. On the other hand, when the values for $U_{Na}V$, C_{Osm}, and weight obtained just before and during steroid administration are plotted against C_{H_2O} (Figure 2) there is a significant correlation between $U_{Na}V$ and C_{H_2O} in all three subjects and between weight and C_{H_2O} in two subjects (Table III). The changes in C_{Osm} were relatively smaller than the changes in C_{H_2O} so that as urine flow increased, the urine became somewhat more dilute in each subject (Figure 2). The rate of potassium excretion showed little change during steroid administration and could not be correlated with any of the other variables measured. Serum electrolyte concentrations also showed little change.

Serum osmolarity varied between 275 and 288 mOsm. per L. before water administration in individual experiments and fell by 5.5 to 14.5 mOsm. per L. after water loading. The changes during steroid administration were of the same magnitude as in the control studies and the mean decrease in all experiments was 10.4 mOsm. per L. or 3.7 per cent of the initial value. This is approximately the change that is to be expected if the water load were evenly distributed throughout the total body water (12, 13). The expansion of plasma volume consequent to water loading, as reflected by change in hemoglobin, hematocrit and serum protein concentration, also appeared to be uninfluenced by steroid administration.

Group II. Acute response to intravenous infusion of hydrocortisone

The acute effects of an intravenous infusion of hydrocortisone were studied in four patients with

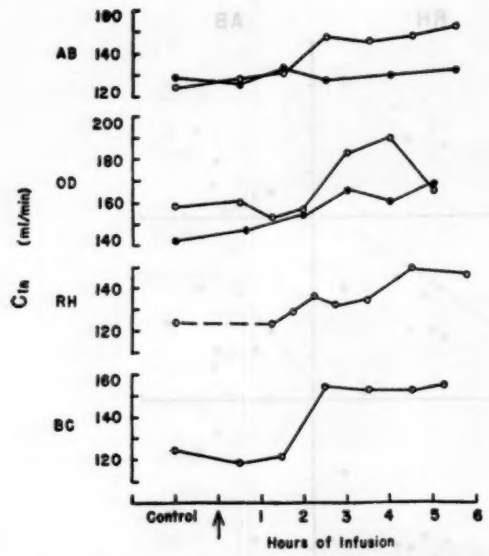

FIG. 3. CHANGES IN GLOMERULAR FILTRATION RATE (C_{In}) DURING HYDROCORTISONE (OPEN CIRCLES) AND CONTROL INFUSIONS (CLOSED CIRCLES)

In Subject R.H., C_{In} was not measured until one-half hour after hydrocortisone infusion was begun. The pre-infusion value in this subject was obtained from a control study done 72 hours previously.

chronic skin disease, but otherwise healthy, while they were receiving a salt-poor diet (three were also the subjects of the studies in Group I-B). An oral water load was established and maintained as in the serial studies. Two hundred mg. of hydrocortisone dissolved in 40 ml. of 50 per cent alcohol were diluted to between 1090 and 1340 ml. with 5 per cent glucose in water and infused at a rate of 3 to 4 ml. per minute. The volume of fluid infused was included in the calculation of water load maintenance. Control observations of water diuresis were made just before the steroid infusion was begun, except in R.H. In this subject the hydrocortisone infusion was given on the first day of the serial study of the effect of prolonged oral steroid administration. In B.C. and A.B. the infusions were given at the end of the post-steroid control period of the serial studies. In A.B. the effect of an infusion containing 20 ml. of 95 per cent alcohol in 1050 ml. of 5 per cent glucose in water and in O.D. the effect of an infusion of 1050 ml. of 5 per cent glucose in water were also determined to provide additional control observations. Urine collections and venous blood samples were obtained at 20 to 60-minute intervals.

A single hydrocortisone infusion had no consistent effect on maximal water diuresis (Table IV, Figure 3). A consistent increase in C_{In} was observed in each subject (Figure 3), beginning during the third hour of infusion. This increase varied between 12 and 27 per cent of the pre-infusion values. During a control infusion of glucose and water alone, there was a rise in C_{In} in subject O.D., but a similar infusion did not affect filtration rate in subject A.B. There was no consistent change in C_{Cr}/C_{In} following hydrocortisone infusion. C_{PAH} rose concurrently with C_{In}. The C_{Urea}/C_{In} ratio did not rise although serum urea values decreased progressively during the infusion.

Changes in sodium and potassium excretion were small. Sodium excretion fell from values of 49 to 75 μEq. per min. to 15 to 35 μEq. per min. during the last part of the hydrocortisone infusion. Potassium excretion was usually slightly higher during the last part of the hydrocortisone infusion than during the first two hours of infusion or during control periods. Serum potassium remained constant, while serum sodium sometimes showed a small decrease. In several experiments, both with and without hydrocortisone, there was a fall in urine flow despite maintenance of the water load. This decrease included a fall in both free-water clearance and osmolal clearance, although the fall in C_{H_2O} was proportionately somewhat greater, so that urine osmolality showed a slight rise.

DISCUSSION

Augmentation of the maximal urine flow during water diuresis was observed in each of nine subjects who were maintained on large doses of adrenocortical hormone. This change must have resulted from an increased rate of glomerular filtration, a decrease in tubular reabsorption of water, or both.[6]

[6] Secretion of water by the tubules (14) could also have been operative, but there is no clear evidence that such secretion occurs. On the other hand there is strong indirect evidence that free-water is made available for excretion, not by tubular secretion, but by reabsorption of solute from isotonic tubular fluid (15). Changes in the rate of absorption of water from the intestine would not account for the observed changes in *maintained* water diuresis, which is little influenced by increasing the magnitude of the water load either orally or parenterally.

TABLE IV

Studies during intravenous infusion of hydrocortisone

Patient, steroid, and fluid admin.	Time	Number of periods	V	C_{H_2O}	C_{Osm}	$U_{Na}V$	U_KV	C_{In}	C_{Cr}	C_{PAH}	C_{Cr}/C_{In}	F.F.	V/C_{In}	C_{Urea}/C_{In}	Total solute: Urine	Total solute: Serum	Serum Na	Serum K
	hours		*ml./min.*			*μEq./min.*		*ml./min.*				*per cent*	*per cent*		*mOsm./L.*		*mEq./L.*	
B. C.																		
200 mg. Cpd. F	Pre-infusion	3	12.5	10.0	2.5	69	104	125	143	656	1.14	19.1	10.0	.67	53	267	135	4.8
in 1,340 ml.	0 to 2	2	10.8	8.7	2.1	42	62	120	130	543	1.08	22.1	9.0	.69	53	266	135	4.6
5% D/W	2 to 5	6	14.5	11.9	2.6	35	82	153	165	740	1.08	20.7	9.5	.68	49	265	131	4.7
R. H.																		
200 mg. Cpd. F	½ to 2	3	21.2	17.9	3.3	57	137	126	153	786	1.21	16.0	16.8	.78	42	274	141	4.3
in 1,100 ml.	2 to 6	5	21.6	18.0	3.6	42	179	141	172	918	1.22	15.4	15.3	.78	43	267	134	4.6
5% D/W																		
O. D.																		
Control	Pre-infusion	3	13.0	10.4	2.6	38	80	142	171	739	1.20	19.2	9.2		54	275	138	5.2
1,150 ml.	0 to 2½	3	11.4	8.9	2.5	35	95	149	164	733	1.10	20.3	7.7		59	272	137	5.1
5% D/W	2½ to 5½	3	10.4	8.1	2.3	61	38	164	177	816	1.08	20.1	6.3		61	270	136	5.1
200 mg. Cpd. F	Pre-infusion	3	15.6	12.6	3.0	75	87	158	172	815	1.09	19.4	9.9		53	274	137	5.1
in 1,150 ml.	0 to 2½	4	12.2	9.5	2.7	49	83	157	167	770	1.06	20.4	7.8		60	273	136	5.0
5% D/W	2½ to 5½	3	12.8	10.0	2.8	29	116	179	189	870	1.06	20.6	7.2		59	272	133	5.1
A. B.																		
Control	Pre-infusion	2	12.8	9.8	3.0	34	176	128	147	628	1.15	20.4	10.0	.61	63	268	130	4.9
20 ml. Ethanol	0 to 2	2	10.8	8.1	2.7	41	105	128	157	615	1.23	20.8	8.4	.64	66	265	129	4.6
in 1,090 ml.	2 to 5½	4	8.2	6.0	2.2	54	54	131	160	685	1.22	19.2	6.3	.62	70	263	130	4.6
5% D/W																		
200 mg. Cpd. F	Pre-infusion	2	13.7	10.5	3.2	49	197	124	173	646	1.40	19.2	11.1	.70	63	271	135	4.8
in 1,090 ml.	0 to 2	2	10.5	7.9	2.6	32	111	129	166	673	1.29	19.2	8.1	.63	67	270	133	4.9
5% D/W	2 to 6	4	9.3	7.0	2.3	13	118	147	187	727	1.27	20.2	6.3	.61	68	271	133	4.9

An increase in glomerular filtration rate during prolonged cortisone or corticotrophin administration has been reported by several observers (16–18). The changes in filtration rate noted during continued hormone administration in the present study do not appear to account for the observed changes in water excretion. Thus, a striking increase in filtration rate occurred during single infusions of hydrocortisone, without augmentation of the rate of water diuresis. Furthermore, during prolonged oral administration of hydrocortisone or cortisone, changes in filtration rate were small, with large changes in the maximal rate of urine flow which could be dissociated temporally from the effect of glomerular filtration rate.

The increase in urine flow during prolonged oral steroid administration was usually accompanied by an increase in total solute excretion. Such an increase in urinary solute is known to augment the diuresis resulting from administration of large water loads (14, 15, 19–22). Moreover, when the magnitude of diuresis in water-loaded subjects is enhanced by increasing the solute load, the concentration of total solute characteristically rises (22, 23). On the other hand the rise in solute output during steroid administration, when it occurred, was so small compared to the increase in urine flow that solute concentration fell. Hence the changes in free water clearance observed cannot be explained on the basis of an increase in solute load with a fixed "maximal urinary diluting power." When dietary sodium was restricted, the increase in urine flow during steroid administration was regularly accompanied by an increase in sodium output[7] (Figure 2). This observation is consistent with the possibility that augmentation of water diuresis might have been related to an increase in the rate of sodium excretion (20). However, this relationship was not regularly observed when the diet was varied (Table I, W.H. and G.S.). Hence, changes in flow could not always be related to change either in the excretion of sodium or of total solute.

Cortisone and hydrocortisone might affect water diuresis by some form of antagonism to antidiuretic hormone (ADH). Excess ADH activity has been proposed as the cause of failure of water diuresis in adrenal insufficiency (4). The evidence is based on bioassays (1, 28) of blood and urine performed by techniques which have been seriously challenged (29). Certainly in the water-loaded normal subject who is in a state of "physiologic diabetes insipidus" it is reasonable to assume that no circulating ADH is present. The validity of this assumption is supported by the observation that in such subjects the administration of alcohol, a powerful inhibitor of ADH, does not enhance urine flow (30), an observation that we have confirmed in a number of subjects, including B.C. of the present study. Therefore, the increased water diuresis observed during steroid administration cannot be ascribed to further inhibition of ADH. We have also observed that the antidiuretic response to small doses of Pitressin® (15 milli-units intravenously) administered during water diuresis is not altered during cortisone administration, so that there is probably no change in the sensitivity of the kidney to ADH.

If the increase in maximal rate of water diuresis during prolonged steroid administration cannot be ascribed to changes in glomerular filtration rate, solute excretion or an effect on ADH, it must be due to an intrinsic change in the renal tubular handling of water. Moreover, the increase is chiefly in free-water clearance. Hence the change must involve the mechanism for formation of "free" water, an operation which is believed to occur in the distal tubule (31–33). In the absence of ADH, the magnitude of free-water clearance is dependent upon the volume of isotonic or possibly hypotonic fluid (34), delivered from the proximal segments, which is rendered free of solute in the distal tubules.[8] According to this schema, the

[7] Although most observers have described a decrease in sodium excretion and a rise in potassium excretion during the administration of cortisone, hydrocortisone, or corticotrophin, the appearance of such changes and their magnitude appear to depend upon the conditions of the experiment (24–27). For example, Liddle, Pechet, and Bartter (26) have noted that in dogs the rate of sodium excretion may rise, fall, or remain constant when adrenocortical hormones are given, depending upon the relative magnitudes of the changes in glomerular filtration rate and sodium reabsorption.

[8] Additional reabsorption of water ($T^c_{H_2O}$) is believed to take place at a still more distal site, presumably the collecting ducts (15). When ADH effect is maximal the distal tubular fluid remains isotonic, so that the further abstraction of water in the collecting ducts results in the formation of a hypertonic urine. Furthermore it appears that $T^c_{H_2O}$ is operative even under conditions of

increase in urine flow and free-water clearance observed during steroid administration could only have been the result of a decrease in proximal solute and water reabsorption, with increased delivery of isotonic fluid to the distal system and increased distal reabsorption of solute without water.

The hypothesis that "free" water is formed in the distal tubule does not require that all of the fluid delivered from the proximal system must be excreted or even delivered to the collecting tubules during water diuresis. Such complete excretion would require absolute impermeability of the distal cells to water in the absence of ADH and free permeability when ADH effect is maximal. It is perhaps more reasonable to suppose that the distal tubular epithelium is always partially permeable to water (33) and that ADH acts to increase the rate of movement of water in response to the concentration gradient established by prior solute reabsorption. Koefoed-Johnson and Ussing (37) have shown that when such an osmotic gradient is established across a piece of isolated frog skin, there is always a net movement of water, but that this movement is more rapid when posterior pituitary extracts are added to the preparation. They suggest that the action of the posterior pituitary extract is to increase the size and/or number of pores in this membrane. If a similar mechanism obtains in the kidney, the increase in free-water clearance during steroid administration could be ascribed to diminution in the permeability of the cell membranes to water. The same rate of solute reabsorption would lead to less water reabsorption and the urine would be more dilute. Such a change might affect all the cells of the renal tubules; certainly localization of the site of action to the distal tubule has not been demonstrated. Lewis (38) has suggested that the failure of water diuresis in adrenal insufficiency and its correction by cortisone administration may be due to inability of the distal tubule to reabsorb solute in excess of water unless an adequate supply of steroid is present. Our findings in normal subjects do not bear directly on this problem, and further studies in patients with adrenal insufficiency are needed.

The augmentation of urine flow and free-water clearance observed during steroid administration may therefore be ascribed to either or both of the following: 1) an increased delivery of solute to the distal tubule with increased reabsorption of solute in this area, 2) a decrease in osmotic permeability to water, so that less water leaves the tubular lumen in response to the osmotic gradient established by solute reabsorption.

The striking changes observed in free-water clearance provide additional evidence against the concept of a relatively constant value for this function (15, 19, 31–33). Other data indicate that an increase in solute excretion during water diuresis may be accompanied by augmentation of free-water clearance (20–22, 34). A fall in free-water clearance associated with declining solute excretion regularly accompanies the decrease in diuresis which occurs when a water load is maintained in seated subjects (20, 23). Variations in diet which are associated with change in the daily load of solute excreted, as well as with change in glomerular filtration rate, also influence the magnitude of water diuresis and of free-water clearance in normal man (22). Striking augmentation of maximal water diuresis occurs in the dog and the seal when an increase in glomerular filtration is induced (39, 40). However, it appears that the administration of adrenocortical hormone, in large doses continued over a number of days, differs from all other procedures known to influence the magnitude of free-water clearance in that its effect does not depend upon change in antidiuretic hormone, glomerular filtration rate, or renal excretion of solute.

During water diuresis the tubular reabsorption of urea is thought to result chiefly from passive back-diffusion in the proximal tubule. The rise in urea/inulin clearance ratio observed in the present studies therefore suggests either a decrease in the proportion of glomerular filtrate reabsorbed in the proximal segment, or a decrease in the permeability of this segment to urea.

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water diuresis (35) so that the amount of free-water actually being formed in the distal system is probably larger than the amount of free-water excreted in the urine by about 5 ml. per min., the maximum value of $T^c_{H_2O}$ in normal man (36). If this moiety of water reabsorption were reduced during steroid administration then *part* of the change observed in free-water clearance could be explained. However, $T^c_{H_2O}$, measured in the hydropenic state during mannitol diuresis, was not altered in one patient whom we studied before and after seven days of intensive cortisone therapy.

SUMMARY

The effects of large doses of cortisone and hydrocortisone on water diuresis and renal function in man have been examined. The rate of urine flow observed during a maintained water load was regularly increased greatly during steroid administration irrespective of changes in the dietary intake and renal excretion of sodium salts.

Serial studies in subjects on a salt-poor diet demonstrated an increase in maximal urine flow and in free-water clearance. The increased excretion of water was usually but not invariably accompanied by a small increase in sodium excretion, a gain in body weight, and sometimes by an increase in total solute excretion. The small rise in glomerular filtration rate noted during steroid administration did not parallel the increase in maximal urine flow.

A single intravenous infusion of hydrocortisone regularly produced an increase in glomerular filtration rate, but without a consistent increase in the diuretic response to water loading.

The observations indicate that continued administration of steroid in large doses influences the renal tubular reabsorption of water. It is suggested that more free water is made available for excretion because of 1) a redistribution of solute reabsorption between proximal and distal systems or 2) an alteration in the permeability of the renal tubular epithelium to water.

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AN ELECTROPHORETIC STUDY OF SERUM PROTEINS IN THYROIDITIS [1]

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The clinical picture of acute thyroiditis [3] may be so classical that the diagnosis is obvious. However, very often, the symptomatology may be obscure. Several investigators have sought for a laboratory test, specific for the disease, to complement the clinical observations. In 1949, Werner, Quimby, and Schmidt (1) reported that six patients with thyroiditis had exceedingly low thyroidal uptakes of radioactive iodine. Hamilton, Kirkendall, and Barker (2), in addition to confirming this isotopic finding, also reported that the level of serum protein bound iodine (PBI) was either high normal or abnormally high. These two laboratory findings have helped to diagnose many obscure cases of thyroiditis. Patients who have ingested or received an injection of iodide compounds, may also show low thyroidal uptake of I-131 and an elevation of the serum PBI. Therefore, it would be helpful to have a test, not dependent on iodine metabolism, confirmatory of the disease.

One year ago, we studied a patient whose fever of unknown origin proved to be due to thyroiditis. In addition to the above mentioned abnormalities in iodine metabolism, we noted a markedly abnormal electrophoretic serum protein pattern. This abnormality consisted of an increase in alpha-1, alpha-2, and gamma globulin. Since this chance observation, we have examined the electrophoretic patterns of the sera of seven other patients who had subacute thyroiditis. All eight electrophoretic patterns were similar. After these data had been assembled, we noted the report of Stemmerman (3) that his four patients with thyroiditis had abnormally high alpha-2 fractions as determined by paper electrophoresis.

It is the purpose of this report to present the electrophoretic patterns of the serum proteins observed in thyroiditis, and to discuss the value of such observations with regard to the diagnosis of this disease. We will also relate the present findings to previous reports of the electrophoretic studies made on pathological sera and thyroid preparations. Finally, some observations will be made on the use of free *versus* paper electrophoresis in clinical diagnosis.

METHODS

Blood was drawn from patients in the fasting state. The separated serum was diluted with three volumes of diethylbarbiturate buffer (pH 8.6, ionic strength 0.1), dialyzed in the cold against the same buffer, and electrophoresed in a Perkin-Elmer model No. 38 Tiselius apparatus. Planimetry of the diagrams established the relative per cent composition of the sera.

The serum protein bound iodine was determined by the dry ashing technique described by Barker, Humphrey, and Soley (4). The range of serum PBI of normal subjects in our laboratory is from 3 to 7 gamma per 100 ml. The uptake of radioactive iodine by the thyroid was measured 24 hours after the ingestion of approximately 5 microcuries of radioactive iodine (I-131). A scintillation counter [4] was placed 20 centimeters from the neck. Counts were taken of the entire head and neck area. The background counts from the head and neck area, excluding the thyroid gland, were then recorded by shielding the thyroid area with a one-inch-thick lead block. The difference between these two counts was considered to be the count from the radioactive iodine within the thyroid gland. Counts were similarly recorded from the contents of a vial containing an aliquot of the ingested dose of I-131 in 10 ml. of water. Thus, the percentage of the ingested dose was easily calculated (5). The range of thyroidal uptakes in normal subjects in our laboratory is between 15 and 50 per cent.

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[3] In this paper we will use the following terms interchangeably to signify the same disease: acute thyroiditis, subacute thyroiditis, and thyroiditis.

[4] Model DS-1 Scintillation Counter, Nuclear Instruments and Chemicals Corp., Chicago, Ill.

RESULTS

Table I contains the data derived from the electrophoretic diagrams. In all eight cases, albumin was lowered from the normal range of 55 to 60 per cent to between 35 to 45 per cent, and gamma globulin was elevated. In seven of the eight cases, there also were marked rises in alpha-1 and alpha-2 globulins. It can be seen that either alpha-1 or alpha-2 globulin may be higher, and that the two may shift their relative concentrations during the course of the patient's disease (Table I, Case 1). In Case 7, alpha-1 globulin, and in Case 8, alpha-2 were not elevated. Case 1 had a quite elevated gamma globulin which decreased over a two-week interval. Slight increases from the normal mean for beta globulin were noted in most of these cases. Cases 1 and 4 were followed for some time after the diagnosis of thyroiditis was established. As the alpha globulins decreased, albumin rose in both, there was an increase in beta globulin in Case 1, while Case 4 had a normal pattern three months later.

Although these eight cases form a small series, the average mean increases from the normal for alpha-1, alpha-2, and gamma globulin are about equal, with a similar standard deviation (Table I).

The thyroidal uptake of I-131 in each of these cases was less than 3 per cent (Table II). Nine of ten other cases of this disease, previously studied in our laboratory, similarly showed a markedly decreased ability to concentrate iodide. The level of serum PBI was above normal in three of the six patients studied and high normal in three others.

DISCUSSION

Significance of electrophoretic data

Our data show that in thyroiditis there occur a consistent elevation of serum alpha-1, alpha-2, and gamma globulins, a less marked rise in beta globulin, and a moderate decrease in albumin. Such changes are not specific for any single disease. Similar findings have been reported for Hodgkin's disease, protein deficiency states, febrile conditions, rheumatoid arthritis, lupus erythematosis disseminata, *etc.* (6–9). Shedlovsky and Scudder concluded from their electrophoretic studies that: "An increase in alpha globulin levels appears to take place, as well as an increase in sedimentation rates, when there is present any considerable inflammation or tissue destruction, irrespective of its cause" (8). They also noted that the highest alpha globulin values corresponded to febrile disease states. Chow demonstrated a definite negative correlation coefficient between the percentages of albumin and total alpha globulins in the various deficiency states studied by him (7). He has also discussed the question of attributing diagnostic specificity to alterations in protein patterns. Petermann, Karnofsky, and Hogness (6) showed that the alpha-1, alpha-2, and gamma globulins in Hodgkin's disease were often elevated to the same extent as we have reported for thyroiditis in this paper. These authors stated: "The electrophoretic

TABLE I

Distribution of serum proteins in thyroiditis

Case	Sex	Age	Date	% Protein in serum					% Deviation from normal		
				Alb.	Alpha-1	Alpha-2	Beta	Gamma	Alpha-1	Alpha-2	Gamma
Normal*				59	5.6	9.3	14.3	12			
1	F	68	10/23/55	35.4	8.04	15	16	25.4	+ 44	+ 62	+111
1	F	68	11/1/55	40.3	8.6	13.3	14.3	23.6	+ 54	+ 44	+ 96
2	M	49	10/31/55	43.5	8.8	14.1	16.5	17	+ 58	+ 52	+ 41
3	F	40	1/11/56	37	11.5	20.5	14.5	16.5	+106	+121	+ 37
4	M	40	2/15/56	42	7.5	17	17.8	15.8	+ 34	+ 83	+ 31
5	M	38	4/20/56	40.6	10.2	15.7	16.1	17.3	+ 83	+ 69	+ 43
6	F	45	4/15/56	42.6	8.8	12.2	17.7	18.5	+ 58	+ 32	+ 53
7	M	48	5/14/56	47	5.8	12.9	17.3	17	+ 4	+ 39	+ 41
8	F	26	11/30/56	43.6	11.0	10.2	15.7	19.3	+ 96	+ 10	+ 61
Mean									+ 60	+ 57	+ 57
Standard deviation									± 30	± 30	± 26

* Modified from Armstrong, S. H., *et al.* (Ref. 12).

TABLE II

Case	Uptake of I^{131}	Serum PBI
1	2*	7.8†
2	3	
3	3	
4	0	5.0
5	0	15.0
6	1	6.9
7	0	5.9
8	2	9.9

* Per cent of ingested dose.
† Gamma per 100 ml. serum.

patterns seen in moderately advanced Hodgkin's disease are consistent with a chronic infectious disease." The bibliography given at the end of the present paper refers to other publications which make abundantly clear the non-specificity of change in the alpha, or gamma globulin content of blood. Skillern, Nelson, and Crile, using free electrophoresis, have studied six cases of thyroiditis. They found a decreased albumin in five of the six, a slightly increased gamma globulin in two, an increased alpha-1 and alpha-2 in one, and a slight elevation of beta globulin in one. They regard the protein changes as non-specific (10).

Stemmerman's apparently incomplete observations in patients with thyroiditis, based on paper electrophoresis, led him to propose a test which we believe is not valid. He did not report any change other than in alpha-2 globulin. Further, he buttresses the significance of his alpha-2 finding by referring to the work of Shulman, Rose, and Witebsky (11). These authors report that crude hog thyroid gland extract was found, by paper electrophoresis, to migrate with a mobility slightly greater than that of hog alpha-2 globulin (1). Reference to this article shows that the mobilities on paper, used to characterize the hog extract, were calculated by extrapolation of data taken from free solution electrophoresis and by reference to albumin migration. The calculated mobility for the hog "thyroglobulin" is an approximation which probably cannot be extrapolated to human sera. In addition, it is well known that all electrophoretic mobilities of proteins are partially dependent on the relative concentration of the protein components in the solution under test (12). This is another reason why the calculations of Shulman, Rose, and Witebsky are of questionable value when applied to the migration of components in human sera, as was done by Stemmerman. This point is clearly demonstrated by Gordon, Gross, O'Connor, and Pitt-Rivers in their electrophoretic study of the nature of the circulating thyroid hormone-plasma protein complex (13). Thyroxine labelled with I-131 was shown to migrate with alpha-1 globulin when mixed with serum, although it did not move from the starting boundary when electrophoresed by itself. These authors also compared free and paper electrophoresis of the same serum and showed the coincidence of the major I-131 activity with the alpha-1 region.

It is evident from the electrophoretic data of the present paper and others cited (6–9) that the pattern found in the sera of patients suffering from thyroiditis is a reflection of a febrile process accompanied by some tissue inflammation and destruction. Perhaps a lack of sensitivity in the paper electrophoresis method used by Stemmerman obscured alpha-1 and gamma globulin changes. Since no quantitative data were given by this author, it is difficult to judge the point. Therefore, it would seem unwise to advocate that paper electrophoresis is a simple substitute for the earlier procedures used in the diagnosis of thyroiditis. A specific laboratory test for thyroiditis still remains to be found.

SUMMARY AND CONCLUSIONS

1. Eight cases of clinically diagnosed thyroiditis have been studied by free solution electrophoresis, radio-iodine uptake, and protein bound iodine determinations.

2. Abnormal serum protein patterns were found in all eight cases. The changes found were considered to be non-specific for the disease, and the result of a febrile process accompanied by some tissue inflammation and destruction. The use of paper electrophoresis as a diagnostic tool in thyroiditis has been evaluated. The claim that an elevated alpha-2 globulin is uniquely characteristic of thyroiditis is not confirmed.

3. The radioactive iodine uptake measurements were significantly abnormal in all eight cases studied.

4. The determination of serum protein bound iodine was less helpful in diagnosing the disease.

5. It is concluded that a specific diagnostic test for thyroiditis does not exist at present.

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THE EQUILIBRATION OF RADIOSODIUM (Na^{24}), RADIOPOTASSIUM (K^{42}), AND DEUTERIUM OXIDE (D_2O) IN HYDROPIC HUMAN SUBJECTS [1]

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Isotope dilution methods have been described for the determination of total exchangeable sodium (Na_e) (1, 2), total exchangeable potassium (K_e) (3–5), and total body water (T.B.W.) (6–8) with Na^{24}, K^{42} and D_2O as tracers. The time required for equilibrium of distribution of each of the isotopes must be determined experimentally, since uniform mixing is an explicit assumption with these methods (9). In normal human subjects the times required for 95 to 100 per cent equilibration were found to be 12 to 20 hours for Na^{24}, 18 to 40 hours for K^{42}, and 2 to 3 hours for D_2O (1–7). The presence of abnormal fluid collections, however, may produce significant delays in diffusion equilibrium.

The present study was undertaken to determine the equilibration time of Na^{24}, K^{42} and D_2O in patients with edema or abnormal fluid collections. Equilibrium of distribution was evaluated by comparisons of specific activities between various body fluids and by determinations of apparent exchangeable quantities as a function of time.

METHODS

A total of 78 hydropic patients, 57 males and 21 females, was studied. Each subject received only one of the three isotopes. The group was divided into patients with congestive heart failure, designated as cardiacs, and those with hepatic cirrhosis and ascites, as well as a few with the nephrotic syndrome, designated as non-cardiacs. Subjects receiving Na^{24} or K^{42} were fed an analyzed diet, containing 10 mEq. of Na and 28 mEq. of K, for the 2 days preceding and the 2 days of the study. Those receiving D_2O were allowed only a dry, low-fat breakfast without fluids at the start of the 6 hours of the procedure and no food or fluids for the period of equilibration.

The isotopes were administered intravenously by calibrated syringe. Two hundred to 400 microcuries of Na^{24} was given to each of 23 patients, 12 cardiac and 11 non-cardiac. Approximately 300 microcuries of K^{42} was given to each of 26 patients, 14 cardiac and 12 non-cardiac. These quantities of isotope are somewhat higher than those ordinarily used in tracer studies because the objectives of the study required prolonged observations. The maximum radiation from Na^{24} was 0.6 r.e.p. and from K^{42} was 0.3 r.e.p. Although these are permissible radiation exposures for two-week periods, lower doses should be used when possible. Forty-five ml. of 99.5 per cent D_2O was given to each of 29 patients, 17 cardiac and 12 non-cardiac.

Venous blood was drawn at 8, 24, 32 and 48 hours following injection of isotonic $Na^{24}Cl$. One-ml. serum aliquots from 6 patients were dried and counted in triplicate with an end-window, sodium-iodide scintillation counter. The probable error of these measurements was ± 1 per cent. In the remaining patients Na^{24} assay was carried out in a well-type sodium-iodide scintillation counter, with a probable error of ± 1 per cent. Transudates were obtained from the thoracic or peritoneal cavities or subcutaneous spaces by aspiration with a needle and syringe simultaneously with venipuncture. The method of measuring radioactivity was the same for each pair of samples.

One- to 2-hour spot urine specimens were collected at 12, 24, 36 and 48 hours following injection of $K^{42}Cl$. Catheterization was used only when necessary to insure complete urine collection. The urine was concentrated to one-third its original volume by heat, and triplicate 1-ml. aliquots of each urine concentrate were dried and counted with an end-window Geiger-Müller tube with a probable error of ± 1.5 per cent. Serum and transudate specimens were obtained at the time that one or more of the spot urine specimens were collected. The protein in these samples was precipitated with 95 per cent ethanol in a ratio of 2 volumes of ethanol to 1 of sample. The supernatants were reduced in volume to approximately 12 ml., and aliquots were taken for assay of chemical potassium and radioactivity. Isotopic assay was performed in triplicate as described above for urine.

All chemical Na and K determinations were done in duplicate with a lithium internal standard flame photom-

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[2] Research Fellow of the National Heart Institute of the United States Public Health Service.

[3] Research Fellow of the American Heart Association.

[4] Established Investigator of the American Heart Association.

TABLE I

THE APPARENT EXCHANGEABLE BODY SODIUM (Na_e) IN PATIENTS WITH EDEMA

Pt. No. Sex	Dx*	Age yrs.	Na_e (mEq) 8 hrs.	24 hrs.	32 hrs.	48 hrs.	Na_e/Body Wt. (mEq./kgm.) 8 hrs.	24 hrs.	32 hrs.	48 hrs.	Na_e/Body Wt. as a Fraction of 32-hr. Value 8 hrs.	24 hrs.	32 hrs.	48 hrs
A. CARDIACS														
1-M	HCVD	54	2510	2746	2793	2798	32.9	43.4	43.8	45.1	0.89	0.99	1.00	1.03
2-F	ASHD	50	5762	6059	6120	5804	67.8	69.8	70.1	65.8	0.97	1.00	1.00	0.94
3-F	ASHD	67	3814	3877	3991	4108	51.8	52.8	54.2	56.3	0.96	0.97	1.00	1.04
4-M	ASHD	52	3139	3114	3104	3102	55.7	56.1	56.8	57.7	0.98	0.99	1.00	1.02
5-M	RHD	56	2382	2386	2412	2442	46.6	46.7	47.4	48.0	0.98	0.99	1.00	1.01
6-M	RHD	53	4149	4340	4410	--	54.5	57.2	58.1	--	0.94	0.98	1.00	--
7-M	ASHD	73	3294	3465	3536	--	61.9	66.3	68.3	--	0.93	0.97	1.00	--
8-M	HCVD	51	5224	5869	5956	5872	54.9	62.4	63.3	63.0	0.87	0.99	1.00	1.00
9-M	ASHD	78	4869	5101	5214	5046	65.3	69.3	70.9	70.2	0.92	0.98	1.00	0.99
10-M	ASHD	73	3294	3426	3449	3506	52.8	56.1	55.6	56.8	0.95	1.01	1.00	1.02
11-F	RHD	50	2992	3025	3079	3083	47.0	47.6	48.7	48.8	0.97	0.98	1.00	1.00
12-M	ASHD	72	3888	4040	4067	4116	53.1	56.6	56.8	58.4	0.93	1.00	1.00	1.03
						Mean:	54.2	57.0	57.8	57.0	0.94	0.99	1.00	1.01
						±S.D.**:	±7.9	±8.7	±8.3	±6.8	±0.04	±0.01	--	±0.03
B. NON-CARDIACS														
13-M	CL	59	3895	4192	4341	4349	57.3	62.8	64.4	65.5	0.89	0.98	1.00	1.02
14-M	DG	61	2642	2774	2908	2819	46.6	49.7	51.7	50.8	0.90	0.96	1.00	0.98
15-M	CL	65	3973	4094	4209	4250	62.3	64.6	66.4	67.6	0.94	0.97	1.00	1.02
16-M	CL	52	5349	5732	5848	6051	81.4	87.2	89.0	92.1	0.91	0.98	1.00	1.03
17-M	CGNS	43	6870	7079	7036	--	83.3	85.8	85.3	--	0.98	1.01	1.00	--
18-M	CL	52	4838	6368	6324	6502	56.0	73.8	74.0	75.7	0.76	1.00	1.00	1.02
19-M	DG	66	4228	4504	4458	4607	59.2	63.6	62.9	66.0	0.94	1.01	1.00	1.05
20-M	CL	55	4397	4562	4607	4713	65.6	67.6	67.8	69.1	0.97	1.00	1.00	1.02
21-M	CL	52	4435	5159	4607	4814	48.2	56.8	50.6	53.2	0.95	1.12	1.00	1.04
22-M	CL	59	2940	3152	3184	3252	42.9	47.2	47.0	48.8	0.91	1.00	1.00	1.04
23-F	DG	55	4100	4112	4091	4088	71.3	71.5	72.2	72.2	0.99	0.99	1.00	1.00
						Mean:	61.3	66.4	66.5	66.1	0.92	1.00	1.00	1.02
						±S.D.**:	±13.3	±12.8	±13.4	±12.9	±0.06	±0.04	--	±0.02
										Mean (combined):	0.93	0.99	1.00	1.02
										±S.D. (combined):**	±0.05	±0.03	--	±0.03

* HCVD = hypertensive cardiovascular disease
ASHD = arteriosclerotic heart disease
RHD = rheumatic heart disease
CL = cirrhosis of liver
DG = diabetic glomerulosclerosis
CGNS = chronic glomerulonephritis, nephrotic stage

** Standard deviation = $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$

eter. A complete urine collection was obtained from each patient during each period of equilibration and analyzed for radioactivity as described. Each patient was weighed at the time of collection of each sample.

Venous blood was collected at 2, 3, 4.5 and 6 hours following injection of sterile 99.5 per cent D_2O containing 0.85 per cent NaCl. Simultaneous transudate specimens were obtained by aspiration at one or more of these times.

TABLE II

THE DISTRIBUTION OF RADIOSODIUM IN TRANSUDATES AND IN SERUM

	Pt. No.	Source of Fluid*	Ratio of Transudate S.A. to Serum S.A. (S.A.R.) 8 hrs.	24 hrs.	32 hrs.
CARDIACS	2	SC	0.73	0.96	1.00
	3	AF	--	1.00	1.01
	4	PF	1.01	1.03	--
	6	SC	--	--	1.01
	7	SC	--	--	1.02
	9	SC	0.76	--	--
	10	SC	--	1.00	--
	11	PF	1.03	--	1.01
NON-CARDIACS	13	AF	0.91	1.01	1.01
	14	SC	0.78	--	--
	15	AF	--	1.00	--
	16	SC	--	0.96	1.00
	17	SC	--	1.00	0.98
	18	AF	0.57	0.99	--
	19	SC	0.84	--	--
	20	AF	0.97	--	1.01
	21	AF	1.00	1.13	--
	22	AF	0.97	0.99	--
		Mean:	0.87	1.01	1.01
		S.D.:	±0.14	±0.04	±0.01

*PF = pleural fluid
AF = ascitic fluid
SC = subcutaneous fluid

Deuterium concentrations were determined in duplicate by the falling drop method (8). Urines were collected and similarly analyzed. The loss of deuterium in the urine did not exceed 1 per cent of the injected dose. The coefficient of variation of replicate determinations of D_2O by this method was found to be 0.6 to 0.9 per cent in a concentration range of 0.10 to 0.20 volumes per cent.

Standard formulas were used in calculating specific activities and Na_e, K_e and T.B.W. (1–9). The total exchangeable quantities, Na_e, K_e and T.B.W., were corrected for isotope excretion in the urine. These corrections were used to calculate the amount of isotope retained at the end of each period allowed for equilibration. Specific activity ratios (S.A.R.) were calculated for transudate: serum in the case of Na^{24}, transudate: urine and urine: serum for K^{42}, and transudate: plasma for deuterium. These ratios were used as a measure of exchange equilibrium (10).

RESULTS

The values for consecutive Na_e determinations with equilibration periods extended to 48 hours are listed in Table I; the last four columns list the values for Na_e per kgm. of body weight, expressed as a fraction of the 32-hour value. The mean of this ratio was 0.99 ± 0.01 at 24 hours and 1.01 ± 0.03 at 48 hours for cardiacs, and 1.00 ± 0.04 at 24 hours and 1.02 ± 0.02 at 48 hours for non-cardiac subjects. In neither series did the Na_e obtained at 24 hours differ significantly from the 48-hour value ($p > 0.05$ [5] for both cardiac and non-cardiac subjects). In 18 of 20 subjects, however, there was an increase in the Na_e/body weight at 48 hours as compared with 24 hours. Combining the two groups gives a mean value of 0.99 ± 0.03 at 24 hours and 1.02 ± 0.03 at 48 hours. The difference between these values does achieve statistical significance ($p < 0.02$). The apparent Na_e at 8 hours did differ significantly from the measurements made after longer equilibration periods.

Transudate: serum S.A.R.'s obtained in 18 patients had a mean value of 1.01 ± 0.04 and 1.01 ± 0.01 at 24 and 32 hours after injection, respectively (see Table II). The specific activity of transudates obtained 8 hours after Na^{24} injection, however, often was considerably lower than that of corresponding sera. These data indicate that

[5] $P > 0.05$ indicates that the probability that these differences are due to chance is greater than 5 in 100. The probability "p" was derived from the "t" test.

TABLE III

THE APPARENT EXCHANGEABLE BODY POTASSIUM (K_e) IN PATIENTS WITH EDEMA

Pt. No.	Dx	Age yrs.	K_e (mEq.)				K_e/Body Wt. (mEq./kgm.)				K_e/Body Wt. as a Fraction of 36-hr. Value			
			12 hrs.	24 hrs.	36 hrs.	48 hrs.	12 hrs.	24 hrs.	36 hrs.	48 hrs.	12 hrs.	24 hrs.	36 hrs.	48 hrs.
CARDIACS														
A. Males														
1K	HCVD	71	1214	1490	1493	1564	19.9	25.0	24.9	26.5	0.81	1.00	1.00	1.05
2K	RHD	67	1688	1961	2259	2319	29.5	34.2	39.7	40.4	0.75	0.87	1.00	1.03
3K	HCVD	69	1906	1999	2120	1957	19.8	20.6	21.8	20.3	0.92	0.94	1.00	0.93
4K	ASHD	81	1212	1344	1369	1429	22.0	24.5	25.2	26.4	0.89	0.98	1.00	1.04
5K	ASHD	64	1324	1588	1791	1780	19.4	23.7	27.9	28.2	0.74	0.89	1.00	1.01
6K	ASHD	67	--	1737	2003	2018	--	29.4	34.1	35.2	--	0.85	1.00	1.00
7K	RHD	24	2345	2720	3211	3326	23.1	26.9	31.9	32.7	0.73	0.85	1.00	1.03
8K	HCVD	61	--	1424	1938	2025	--	18.5	25.2	26.6	--	0.74	1.00	1.05
						Mean:	22.3	25.4	28.8	29.5	0.81	0.89	1.00	1.02
						S.D.:	±3.8	±4.9	±5.7	±6.1	±0.08	±0.08	--	±0.04
B. Females														
9K	HCVD	83	--	1350	1406	1303	--	25.2	26.4	25.1	--	0.96	1.00	0.96
10K	ASHD	60	1090	1574	1928	1958	15.1	21.7	27.1	27.4	0.57	0.82	1.00	1.01
11K	RHD	48	1580	1853	2049	2105	18.9	21.9	24.2	24.9	0.78	0.91	1.00	1.03
12K	ASHD	67	923	1067	1344	1441	19.5	22.6	28.7	31.3	0.69	0.79	1.00	1.07
						Mean:	17.8	22.9	26.6	27.2	0.68	0.87	1.00	1.02
CIRRHOSIS OF THE LIVER AND ASCITES														
A. Males														
13K		65	1130	1698	2146	2150	15.3	23.2	29.2	29.4	0.53	0.78	1.00	1.01
14K		52	1203	1437	1574	1642	16.1	19.2	21.0	22.6	0.78	0.91	1.00	1.04
15K		50	1436	2238	2318	2302	19.4	31.0	32.1	32.2	0.62	0.97	1.00	1.00
16K		54	1507	1596	1613	1711	23.6	24.9	25.5	27.0	0.93	0.99	1.00	1.06
17K		57	1615	1731	1796	1953	25.2	26.6	28.5	31.1	0.93	0.96	1.00	1.09
18K		55	1635	1873	1920	2074	25.3	28.9	29.7	32.4	0.87	0.98	1.00	1.07
19K		66	1553	1723	1831	1868	20.8	23.0	25.1	25.7	0.85	0.94	1.00	1.02
20K		59	1371	1655	1884	--	25.2	30.2	34.8	--	0.73	0.88	1.00	--
21K		74	1952	2267	2454	2552	16.8	19.5	21.2	22.2	0.80	0.92	1.00	1.04
						Mean:	20.9	25.2	27.5	27.9	0.78	0.93	1.00	1.04
						S.D.:	±4.0	±4.3	±4.7	±4.2	±0.14	±0.07	--	±0.03
B. Females														
22K		36	1326	1462	1465	1592	24.2	26.7	26.5	28.7	0.91	1.00	1.00	1.09
23K		38	1264	1450	1675	1642	20.7	23.6	27.9	26.9	0.75	0.87	1.00	0.96
						Mean:	22.5	25.2	27.2	27.8	0.83	0.93	1.00	1.02
									Mean (combined):		0.78	0.90	1.00	1.03
									S.D. (combined):		±0.11	±0.07	--	±0.04

TABLE IV
THE DISTRIBUTION OF RADIOPOTASSIUM IN TRANSUDATES, IN SERUM AND IN URINE

	Pt. No.	Ratio of Spot Urine S.A. to Serum S.A. (S.A.R.)		Source of Fluid*	Ratio of Transudate S.A. to Spot Urine S.A. (S.A.R.)			
		12 hrs.	24 hrs.		12 hrs.	24 hrs.	36 hrs.	48 hrs.
CARDIACS	1K	--	1.11	PF	1.09	--	0.93	--
	2K	--	--	AF	1.05	1.06	1.02	--
	3K	1.02	--	AF	1.04	0.98	0.90	--
	4K	--	0.90	AF	--	1.15	--	1.07
	5K	--	1.13	PF	0.93	1.01	0.93	0.93
	6K	--	0.92	PF	--	1.30	1.17	--
	7K	--	0.90	---	--	--	--	--
	8K	--	1.03	PF	--	--	0.99	0.96
	9K	--	0.97	PF	--	0.88	--	--
	10K	--	0.91	---	--	--	--	--
	11K	1.60	--	PF	0.88	0.98	--	1.11
	12K	--	1.00	AF	1.22	1.13	1.13	1.04
		--	1.19	PF	--	--	1.04	1.00
NON-CARDIACS	1K	--	0.85	---	--	--	--	--
	13K	1.48	1.08	AF	0.99	1.12	1.12	1.04
	14K	--	1.12	AF	1.06	--	1.01	--
	15K	1.24	--	---	--	--	--	--
	16K	0.93	--	AF	1.01	0.96	--	--
	17K	1.07	--	AF	1.43	1.23	1.08	--
	18K	0.97	--	AF	1.23	1.01	1.01	--
	19K	--	0.94	AF	1.16	--	--	1.04
	20K	--	0.96	---	--	--	--	--
	21K	--	0.98	AF	--	1.04	1.07	0.96
	22K	1.04	--	AF	1.05	1.03	--	--
	23K	--	0.98	AF	--	--	1.16	1.02
	2K	--	1.01	AF	--	1.06	1.00	--
	Mean:	1.11	1.00		1.09	1.06	1.04	1.02
	S.D.:	±0.18	±0.09		±0.14	±0.11	±0.08	±0.06

*PF = pleural fluid
AF = ascitic fluid
SC = subcutaneous fluid

equilibration of Na^{24} is substantially complete at 24 hours in these hydropic patients. The values for Na_e per kgm. of body weight were considerably higher than normal, confirming previous reports of a positive correlation between edema and body sodium content (11–13).

The results of the K^{42} study are listed in Tables III and IV. The K_e values listed in Table III were calculated from spot urine specific activities. The apparent K_e per kgm. of body weight for the combined series, calculated as a fraction of the 36-hour value, was significantly smaller at 24 hours than either the 36- or 48-hour value ($p < 0.02$). In cardiac patients the mean K_e relative to the 36-hour value was 0.88 ± 0.08 at 24 hours and 1.02 ± 0.05 at 48 hours. In patients with cirrhosis of the liver and ascites, the corresponding values were 0.93 ± 0.07 at 24 hours and 1.04 ± 0.04 at 48 hours. The magnitude of the changes in K_e from 24 to 48 hours in both groups of subjects is too large to be ascribed to any possible contribution from dietary potassium.

Comparisons of simultaneous serum : urine specific activities were obtained to validate the use of urine samples as representative of K^{42} specific activity in body fluids. The data listed in Table IV indicate that the specific activities are identical after 24 hours of isotope equilibration. The scatter in these data probably results from the lesser accuracy of serum assays because of the lower counting rates of these samples. The transudate : spot urine S.A.R. was high during the early part of the equilibration period and declined progressively to mean values of 1.04 ± 0.08 at 36 hours and 1.02 ± 0.06 at 48 hours. The combined mean values for relative K_e show only a 3 per cent rise between 36 and 48 hours. It appears, therefore, that K^{42} equilibrates almost completely between 36 and 48 hours in hydropic patients. It has been shown in normal man, in rabbits and in rats that about 40 hours are required for uniform tissue distribution of K^{42}, except for erythrocytes and brain (3, 14). The low values of K_e per kgm. of body weight in hydropic patients are in accord with previous studies (13, 15).

Tables V and VI list the results of studies on the equilibration of D_2O in patients with edema and ascites. The dilution of D_2O reached a plateau 3 to 6 hours after injection in both groups of patients (see Table V). The 6-hour T.B.W. dif-

TABLE V

THE APPARENT TOTAL BODY WATER (T.B.W.) IN PATIENTS WITH EDEMA

Pt. No.	Dx	Age yrs.	T.B.W. (liters)				T.B.W./Body Wt. (liters/kgm.)				T.B.W./Body Wt. as a Fraction of 4.5-hr. Value			
			2 hrs.	3 hrs.	4.5 hrs.	6 hrs.	2 hrs.	3 hrs.	4.5 hrs.	6 hrs.	2 hrs.	3 hrs.	4.5 hrs.	6 hrs.
CARDIACS														
A. Males														
1D	ASHD	72	42.5	42.0	42.3	42.6	67.1	66.4	66.8	67.1	1.00	0.99	1.00	1.00
2D	ASHD	55	43.1	42.7	43.1	42.2	62.4	61.8	62.3	61.1	1.00	0.99	1.00	0.98
3D	ASHD	50	35.9.	36.2	34.2	39.2	52.7	53.0	50.1	57.5	1.05	1.06	1.00	1.15
4D	ASHD	82	37.7	38.7	39.3	39.6	65.4	67.0	68.1	68.6	0.96	0.98	1.00	1.01
5D	ASHD	73	35.4	36.0	36.0	34.7	60.8	62.1	61.8	59.6	0.99	1.01	1.00	0.97
6D	HCVD	47	45.3	44.4	45.6	43.3	49.1	48.0	49.4	47.1	0.99	0.97	1.00	0.95
7D	HCVD	78	36.7	37.6	37.1	38.9	60.1	62.1	60.7	64.0	0.99	1.02	1.00	1.05
8D	RHD	67	34.8	35.0	36.4	35.8	62.2	62.6	65.3	63.6	0.95	0.96	1.00	0.97
9D	Unknown Etiology	54	25.4	26.2	26.3	26.0	60.1	62.7	63.5	63.7	0.95	0.99	1.00	1.00
10D	ASHD	64	47.6	48.6	47.6	49.6	67.3	68.9	67.7	70.9	0.99	1.02	1.00	1.05
11D	ASHD	61	--	59.9	60.1	--	--	77.2	77.4	--	--	1.00	1.00	--
						Mean:	60.7	62.9	63.0	62.3	0.99	1.00	1.00	1.02
						S.D.:	±5.7	±7.7	±7.9	±6.7	±0.03	±0.03	--	±0.06
B. Females														
12D	RHD	75	30.2	30.6	30.2	30.4	76.1	77.1	76.0	76.5	1.00	1.01	1.00	1.01
13D	ASHD	79	28.5	29.2	29.4	29.4	49.1	50.3	50.6	50.6	0.97	0.99	1.00	1.00
14D	RHD	67	37.4	40.0	39.9	40.4	56.4	60.3	60.4	61.4	0.93	1.00	1.00	1.02
15D	ASHD	70	23.7	38.7	41.1	41.4	43.2	70.5	74.8	75.4	0.58	0.94	1.00	1.01
16D	ASHD	70	39.0	39.7	41.1	42.2	57.7	58.6	60.7	62.4	0.95	0.97	1.00	1.03
17D	ASHD	71	--	66.3	65.9	66.3	--	68.7	68.2	68.6	--	1.01	1.00	1.01
						Mean:	56.5	64.3	65.1	65.8	0.89	0.99	1.00	1.01
						S.D.:	±12.4	±9.7	±9.5	±9.8	±0.17	±0.03	--	±0.01
NON-CARDIACS														
A. Males														
18D	NS*	43	51.5	53.3	54.5	56.4	69.4	71.8	73.4	76.0	0.95	0.98	1.00	1.04
19D	CL	56	37.3	37.1	37.5	37.8	61.6	61.3	61.8	62.4	1.00	0.99	1.00	1.01
20D	CL	60	50.2	50.1	53.6	52.2	62.7	62.6	66.6	64.9	0.94	0.94	1.00	0.97
21D	DG	66	38.7	38.0	39.3	39.2	61.7	60.6	62.5	62.8	0.99	0.97	1.00	1.00
22D	Hem*	66	43.7	45.5	49.0	49.0	59.6	62.2	66.9	66.8	0.89	0.93	1.00	1.00
23D	CL	55	--	37.4	38.0	38.0	--	64.2	65.1	65.2	--	0.99	1.00	1.00
24D	Ans*	54	25.4	--	26.9	27.7	67.3	--	70.8	73.6	0.95	--	1.00	1.04
25D	CL	57	36.2	36.2	36.8	37.7	57.4	57.2	58.6	60.1	0.98	0.98	1.00	1.03
						Mean:	62.8	62.8	65.7	66.5	0.96	0.97	1.00	1.01
						S.D.:	±4.2	±4.5	±4.8	±5.6	±0.04	±0.02	--	±0.02
B. Females														
26D	CL	40	31.9	33.8	35.7	34.3	53.7	56.8	59.9	57.6	0.90	0.95	1.00	0.96
27D	CL	55	33.8	34.8	34.2	33.9	60.0	62.6	61.5	61.0	0.98	1.02	1.00	0.99
28D	CL	36	33.3	33.3	33.7	--	59.0	59.3	59.9	--	0.98	0.99	1.00	--
29D	CL	53	32.6	32.6	--	--	50.0	50.0	--	--	--	--	--	--
						Mean:	55.7	57.2	60.4	59.3	0.95	0.99	1.00	0.98
										Mean (combined):	0.95	0.99	1.00	1.01
										S.D. (combined):	±0.09	±0.03	--	±0.04

*NS = Nephrotic Syndrome Hem = Hemochromatosis Ans = Anasarca, ? Nephrotic

TABLE VI

THE DISTRIBUTION OF D_2O IN TRANSUDATES AND IN SERUM

	Pt. No.	Source of Fluid*	Ratio of Transudate D_2O Concentration to Serum D_2O Concentration (S.A.R.)			
			2 hrs.	3 hrs.	4.5 hrs.	6 hrs.
CARDIACS	1D	PF	0.68	0.79	0.90	--
	2D	PF	0.92	0.98	--	--
	3D	PF	0.94	0.91	--	--
	4D	SC	0.46	0.59	0.71	--
	6D	SC	1.08	1.00	1.07	1.03
	7D	PF	--	--	0.83	0.94
	8D	AF	0.94	0.97	1.02	1.00
	9D	SC	--	--	0.83	0.93
	10D	PF	0.73	0.84	0.88	0.98
	11D	PF	--	--	0.99	--
	12D	PF	0.95	1.02	--	--
	14D	PF	0.56	--	--	--
	15D	PF	0.47	0.86	--	0.99
	17D	AF	--	1.01	1.01	--
NON-CARDIACS	18D	SC	0.77	0.92	--	--
	19D	AF	1.03	0.99	0.99	--
	21D	SC	0.31	--	--	0.95
	22D	AF	0.66	0.81	0.92	0.96
	23D	AF	--	0.89	1.01	1.01
	24D	AF	1.05	--	1.06	1.01
	25D	AF	--	0.84	0.93	0.97
	26D	AF	0.75	--	1.01	0.97
	27D	AF	1.03	1.06	1.07	--
	28D	AF	0.96	1.00	0.99	--
	29D	AF	--	0.98	--	--
		Mean:	0.79	0.91	0.95	0.98
		S.D.:	±0.23	±0.11	±0.09	±0.03

*PF = pleural fluid
AF = ascitic fluid
SC = subcutaneous fluid

fered significantly from the 4.5-hour value in only one instance (patient 3D). The 6-hour T.B.W. value in this case was more than three standard deviations from the combined mean.

Equilibration of D_2O between transudates and serum averaged only 0.91 ± 0.11 at 3 hours. Equilibration in transudates was substantially complete at 6 hours (see Table VI). These data also indicate uniform equilibration in each case at 6 hours, since the range of S.A.R. values at this time was only 0.93 to 1.03. Although calculated T.B.W. showed little change between 3 and 6 hours, the distribution of D_2O in transudates indicates that 4.5 to 6 hours are required for D_2O equilibration in hydropic patients. Total body water expressed per kgm. of body weight is considerably higher in these subjects than in normal subjects of the same age and sex (7).

DISCUSSION

Isotope equilibration could be delayed for a number of reasons in patients with abnormal states of hydration. Diffusion equilibrium could be prolonged because of increased mean diffusion distances or because of decreased surface: volume ratios in various regions of the body (16). In patients with cardiovascular disease and edema, blood flow per unit volume of tissue water might be reduced sufficiently to produce some delay in uniform distribution of isotope.

Two techniques were used in this study to evaluate equilibration of Na^{24}, K^{42}, and D_2O in the presence of abnormal fluid collections. The apparent exchangeable quantity determined as a function of time was used as such a measure on the assumption that during short intervals (*i.e.*, 48 hours) there is little change in body composition. The partition of isotope between serum and transudates was a further check on distribution equilibrium. Since the standard error of the methods for determining either exchangeable quantities or S.A.R.'s approximates 1 per cent, agreement within 5 per cent between simultaneous or consecutive values has been taken as indicative of complete equilibration within 95 per cent confidence limits for any pair of values.

Radiosodium is almost uniformly distributed in its exchangeable mass in 12 to 20 hours in normal man (2, 17, 18). Exchangeable sodium does not include approximately 15 per cent of body

sodium which is sequestered in bone (10). There appears to be an additional 6 per cent of body sodium which exchanges slowly and does not reach a plateau until 96 hours after injection. This slowly exchanging fraction is probably a part of the exchangeable bone sodium pool (19). Because of safety requirements it is more prudent to use Na^{24} with its short half-life and short equilibration periods (*i.e.*, 24 hours) than to use Na^{22} and longer equilibration periods in human subjects. The data in Tables I and II indicate that radiosodium equilibration is almost complete after 24 hours in patients with edema either of cardiac or non-cardiac origin. There is a slight upward trend in relative Na_e from 24 to 48 hours, but to precisely the same extent as in normally hydrated subjects (19). Hemodynamic factors have little influence, since the patients with congestive heart failure did not differ from those with liver or kidney disease in this respect. Although the data were not reported, Miller and Wilson (18) stated that radiosodium exchange is not complete until 24 hours after injection in cases of pleural effusion. Our findings confirm this statement (see Tables I and II).

There have been no specific studies of K^{42} equilibration in hydropic subjects. Corsa, Olney, Steenburg, Ball, and Moore (3), on the basis of tissue studies in rabbits and in patients without edema, estimated that 95 per cent of body potassium exchange in 40 hours, with red cells, brain and bone constituting slowly exchanging pools. The data in Table IV demonstrate that the potassium in abnormal fluid collections reaches equilibrium with radiopotassium in 36 to 48 hours. Furthermore, the K_e values determined from spot urine specific activities reach plateau values between 36 and 48 hours (see Table III). These results indicate that the rate of potassium equilibration is approximately the same in edematous and normally hydrated subjects. The short half-life of K^{42} makes it impractical to extend the time allowed for equilibration beyond 40 to 48 hours. The finding of low K_e per kgm. of body weight in edematous subjects confirms similar observations based on shorter equilibration periods in previous reports (13, 15). It is not certain that there is a reduction in K_e in relation to lean body mass in patients with congestive heart failure or cirrhosis of the liver, since body water content is increased in these subjects and body fat content may be reduced as well.

Four and one-half to 6 hours are required for uniform distribution of D_2O in transudates (see Table VI). Relative T.B.W. reaches a plateau at 3 hours (see Table V). Hurst, Schemm, and Vogel (20) found that in 4 of 20 hydropic patients the transudate : plasma DHO ratio did not reach 4 per cent agreement by 6 hours. Faller, Petty, Last, Pascale, and Bond (21) found that 9 of 14 hydropic patients required more than 6 hours for deuterium equilibration between serum and transudate. Indwelling needles were used for fluid collections in the latter study. Their results may have been influenced by stasis and inflammatory reaction induced by leaving needles in the tissues for many hours. In the subjects we studied D_2O distribution equilibrium occurred in 6 hours, even in the presence of massive fluid collections.

SUMMARY

The equilibration of Na^{24}, K^{42} and D_2O was studied in hydropic subjects. The equilibration of Na^{24} with the readily exchangeable sodium requires at least 24 hours, the equilibration of K^{42} requires 48 hours, and that of D_2O, 6 hours in these subjects. Approximately 94 per cent of exchangeable sodium, 95 per cent of exchangeable potassium and 100 per cent of total body water are measured with equilibration periods as detailed above.

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THE RESPONSE OF RHEUMATIC AND NON-RHEUMATIC CHILDREN TO STREPTOLYSIN O CONCENTRATE [1]

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The observation that individuals who acquire rheumatic fever following a streptococcal infection usually respond with higher streptococcal antibody titres than those who have streptococcal infections uncomplicated by rheumatic fever has been reported repeatedly (1–7). There are exceptions, but, if rheumatics are considered as a group, this observation seems to hold true and it has led to an assumption that rheumatic individuals, that is, those who acquire rheumatic fever or are especially susceptible because one or both parents, a sibling or near relative has had rheumatic fever, respond in a different manner to streptococcal infections than non-rheumatic persons. The response of rheumatic and non-rheumatic subjects to antigenic stimuli has been the objective of several recent investigations (8–11) none of which has shown any significant differences between rheumatics and non-rheumatics in their response to a variety of *non-streptococcal* antigens with the exception that when a strain of *Brucella abortus* was used as the antigen (12, 13) rheumatic subjects developed higher levels of agglutinins and incomplete antibodies than normal persons. However, the more crucial question would seem to be: "Do rheumatics respond differently to *streptococcal* infections or antigens than non-rheumatics?" To conclude that rheumatic individuals respond with higher streptococcal antibody titres than non-rheumatics following a streptococcal infection because they are fundamentally different is open to a number of criticisms, one of which is that the dose of antigen in a streptococcal infection is unknown and it could be that some patients have higher antibody titres because they received a larger dose of antigen during the infection. It is the purpose of this paper to report an investigation of the problem of whether rheumatic individuals respond to equivalent doses of streptococcal antigens in a different manner than non-rheumatic individuals.

METHODS

Preparation of streptolysin O concentrate. A modification of a method for purification of streptolysin O by Herbert and Todd (14) was used to prepare the concentrated streptococcal antigens. The starting material was the filtrate from 5 to 6 litres of beef heart infusion broth prepared from fresh beef hearts, in which a strain of group A type 3 beta hemolytic streptococcus had grown for 18 hours at 37°C. Six lots of streptolysin O were prepared, the end-products of three preparations containing the most highly concentrated streptolysin O being combined. The steps in the purification procedure were the same as those outlined by Herbert and Todd with the exception that the final step of adsorption and elution from alumina C gamma gel was not performed. The final products were dry, fluffy, light tan to dark brown in color with some stickiness in two of the preparations. The yields were 26, 102, 35, 208, 187 and 280 mg.

Test for streptolysin O combining power. Five mg. of streptolysin O concentrate were dissolved in 1.0 ml. M/15 phosphate buffer pH 6.5 containing 1.5 mg. L-cysteine HCl per ml. just prior to performing the test. The test was performed in a manner similar to that of Hodge and Swift (15). The mixed preparation used as antigen contained approximately 400 combining units per ml. and for purposes of calculating equivalent doses was fixed arbitrarily at 400 combining units per ml. or 80 per mg. The preparation of 12/31/54 had 100 combining units per ml. and the preparation of 1/10/55 contained 12.5 combining units per ml.

Test for the presence of streptokinase in the streptolysin O concentrate. The method of Kaplan and his associates (16) was used to test for the presence of streptokinase. The preparation of 1/10/55 showed complete clot lysis at a dilution of 1:4 and no lysis at 1:8 indicating the presence of a small amount of streptokinase. The mixed preparation was not actually tested for streptokinase but since it was prepared from the same strain of streptococcus it is assumed that it also contained a small amount of streptokinase.

Test for the presence of hyaluronidase in the streptolysin O concentrate. A modification (17) of the method of McLean (18) was employed to test for hyaluronidase. In the preparation of 1/10/55 there was no hyaluronidase

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TABLE I

Antistreptolysin O levels in rabbits after streptolysin O administration *

	Rabbit No.	1	2	3	4
	Dose and route of administration	10 units Sl"O"† intravenous q 3 days × 9	40 units Sl"O" intraperitoneal q 3 days × 9	40 units Sl"O" intravenous q 3 days × 9	10 units Sl"O" intraperitoneal q 3 days × 9
Day of experiment	0	0	0	0	0
	16	0	50	0	20
	28	Last dose of antigen	Last dose of antigen	Last dose of antigen	Last dose of antigen
	31	125	125	62.5	250
	54	40 units Sl"O" intradermal booster			40 units Sl"O" intradermal booster
	54	25			62.5
	65	25			62.5
	106	80 units intramuscular	80 units intraperitoneal	80 units intraperitoneal	80 units intramuscular
	106	25	25	25	12.5
	121	83.3	25	25	>159

* Numbers = units of antistreptolysin O.
† Sl"O" = streptolysin O.

and therefore it is assumed there was no hyaluronidase in any preparation.

Preparation of streptolysin O concentrate for injection into rabbits and humans. The work of Todd (19) indicated that streptolysin in the reduced state is a better antigen than when not reduced; therefore, 1.5 mg. L-cysteine HCl per ml. were added so that the streptolysin would be in a reduced state when injected. This vaccine contained approximately 40 combining units of streptolysin O, 500 micrograms of protein or 80 micrograms nitrogen per 0.1 ml. of vaccine.

Test for antigenicity of the streptolysin O concentrate. In order to determine if the streptolysin O concentrate was antigenic, four young adult rabbits were injected by the routes and in the doses shown in Table I. It proved to be a very good antigen as shown by the antibody response which ranged from initial levels of zero to post immunization levels of 62.5 to 250 units of antistreptolysin O. The anticipated administration of the vaccine to humans was considered to be in the nature of a "booster" dose, since probably all of the human subjects would have had previous infections. Therefore, an attempt was made to determine the best route of injection of a booster dose in these rabbits to obtain the maximum antibody response. There was no response at all when the booster dose of antigen was administered intradermally or intraperitoneally but a very good response, from 25 to 83.3 and 12.5 to over 159 units of antistreptolysin O in two rabbits, when the route was intramuscular.

Route of injection and dose of streptolysin O concentrate in experimental subjects. Before giving the streptolysin O concentrate to children, the author injected 80 units intradermally into his right forearm. The next day, a large, tender, bright red, raised, swollen, indurated area 6 × 11 cm. was present about the injection site. Lymphangitis and lymphadenitis were present with malaise and fever. The area of erythema increased in size over a 2-day period and then gradually faded. Lymphangitis and lymphadenitis also gradually disappeared. The entire area desquamated after 2 weeks. (A small scar 4 to 5 mm. in diameter remains at the injection site.) Two other adult volunteers E. B. and R. H. received 8 and 16 units, respectively, intradermally, with similar but milder local and systemic reactions. After these experiences it was decided not to give any of the antigen intradermally but rather to use the intramuscular route.

The dose of streptolysin O concentrate was established arbitrarily according to the age of the subject. Children below the age of ten received 40 units with the exception of one large 10-year-old who received 60 units and two who received 8 units. Five non-rheumatics 11 years or older received 60 or more units, 16 received 40 units, 2 received 16 and 1 received 8 units. All but 3 of the rheumatic children 12 years or older received 60 units, the remaining 3 received 40, 50 and 80 units.

Rheumatic subjects—20 children. Average age—10.8 years. None of these children had an upper respiratory infection within 2 months prior to receiving the antigen or for at least 3 weeks after the antigen. Characteristics of this group are shown in Table II.

Non-rheumatic subjects—55 children. Average age—10.9 years. These normal children with no historical or physical evidence of rheumatic fever or rheumatic heart disease all received antigen. None had an upper respiratory infection of any kind for at least a month before or three weeks after receiving antigen and their throat cultures were negative for hemolytic streptococci.

Non-rheumatic children—45 children. Average age—11.8 years. These non-rheumatic children served as additional controls and received no antigen. Thirteen children had no historical or clinical evidence of an upper respiratory infection within one month previous to the period of observation or during the three-week period of observation.

Thirty-two children had historical or clinical evidence of an upper respiratory infection one month previous to

TABLE II

Rheumatic subjects

Name	Age	Sex	Race	Diagnosis*	Difference in Asl"O"† titre	Dose of Sl"O"‡	Reaction to antigen
D. L.	6	M	W	R.F. Inactive	+1	40	Mild local tenderness.
L. P.	6	F	W	R.F. Inactive	+3	40	None
L. F.	7	F	C	R.H.D. Inactive with MI	+1	40	Mild local reaction.
F. G.	7	M	W	R.H.D. Inactive with MI	+1	40	Edema, entire arm, tenderness, erythema, induration 3–4 cm. about injection. T. 104°F. Fever 3–4 days.
W. P.	8	M	W	R.F. Inactive	+1	40	Edema, entire arm, tenderness, erythema, induration 8–10 cm. 3–4 days, T. 100°F 2–3 days.
J. B.	9	M	W	R.H.D. Inactive with MS	+3	40	None
R. R.	10	M	C	R.F. Inactive	+1	40	Local edema, tenderness, erythema. T. 102°F Malaise.
R. A.	10	M	W	R.H.D. Inactive with MI	+1	40	Sore arm 3 days.
M. H.	11	F	C	Chorea, probably inactive	0	40	Mild local tenderness.
G. J.	11	F	W	R.H.D. ?Inactive MI	0	40	Local edema and tenderness 2 days.
R. L.	12	M	W	R.F. Inactive	+1	60	Severe local reaction with induration, tenderness, erythema 8–10 cm. T. 102°F 2 days.
W. P.	12	M	W	R.F. Inactive with ?MI	+6	60	Severe local reaction, swollen, tender arm. T. 104°F. Fever 4 days. Malaise 4–5 days. Arthritis.
E. E.	12	M	W	R.F. Probably inactive	0	60	Mild local tenderness.
M. J. B.	12	F	W	R.F. Inactive	0	60	Local erythema, tenderness, induration, lymphadenopathy, nausea, vomiting, T. 102°F.
R. J.	13	M	W	Chorea, Inactive R.H.D. with AI and MI	−1	50	Local tenderness 2 days.
M. K.	13	F	C	R.H.D. ?Inactive MS, MI	+1	60	Local tenderness 3 days.
P. W.	14	M	W	R.F. Inactive	+1	60	Local edema and tenderness 2 days.
M. J.	14	F	C	R.H.D. Active with MI	+4	60	Mild local tenderness 1 day.
S. H.	15	F	W	R.H.D. Inactive with MI	+1	60	None
C. S.	16	M	W	R.F. Inactive	+3	80	Local tenderness 2 days.

* R.F. = Rheumatic Fever; R.H.D. = Rheumatic Heart Disease; MI = Mitral Insufficiency; MS = Mitral Stenosis; AI = Aortic Insufficiency.

† Increase, decrease or no change in antistreptolysin O titre by tube dilutions.

‡ Streptolysin O concentrate in combining units.

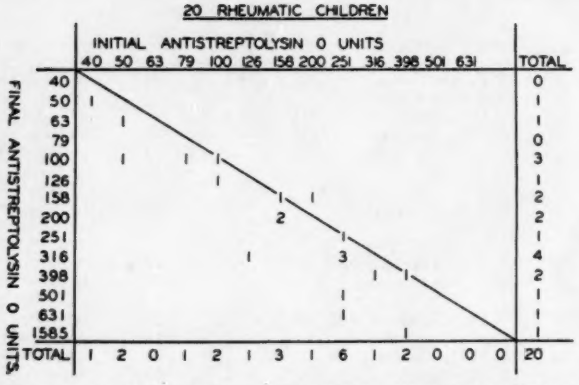
the period of observation or during the three weeks of observation. Ten of these children received penicillin prior to the first examination. Throat cultures of 12 of these children were positive for hemolytic streptococci either at the time of examination or 3 weeks later. In three children there was clinical evidence of a throat infection at the time of examination.

A history was taken to determine if the subject ever had rheumatic fever. A physical examination was performed before the injection of antigen and 3 weeks later with special attention to the nasopharynx for clinical evidence of infections and to the cardiovascular system for evidence of rheumatic heart disease. The rheumatic children were all being followed in the Pediatric Heart Clinic of Vanderbilt Hospital and were all on sulfadiazine prophylaxis. Throat cultures were taken on every child at the beginning and again at the end of the three-week observation period. Other laboratory examinations of the rheumatic children included urinalysis, white blood cell count, packed cell volume, erythrocyte sedimentation rate, and C-reactive protein. Electrocardiograms were done only on four children. Blood specimens were drawn and laboratory examinations performed just before the antigen was given and again 21 days later. The serum was separated, merthiolate added to a concentration of 1:10,000 and stored at 4°C. Each serum specimen was tested for antistreptolysin O, antistreptokinase and antihyaluronidase.

Serological methods

Antistreptolysin O. The method of Hodge and Swift (15) was used. Each serum antistreptolysin O titre was determined in duplicate by making one stock solution of 1:50 dilution and determining the titre of the stock dilution in duplicate tests on the same day. The greatest care was taken to eliminate any possible source of error. Sera were heated at 56°C. for 30 minutes before the test. The serum dilutions were increased in log units by a factor of 0.1. Identical results were obtained from the duplicate titrations. In addition, antistreptolysin O determinations on the rheumatic sera were made on different portions of each serum obtained from blood drawn at the same time. These determinations were done by a different technician at a different time in a different re-

ANTISTREPTOLYSIN O TITERS OF BLOOD SERUM BEFORE AND 3 WEEKS AFTER INJECTION OF STREPTOLYSIN O CONCENTRATE



AVERAGE TITER BEFORE INJECTION = 2.20 ± .07 (LOG UNITS) = 158 COMBINING UNITS
AVERAGE TITER AFTER INJECTION = 2.34 ± .08 (LOG UNITS) = 221 COMBINING UNITS
AVERAGE RISE = 0.145 ± .038 (LOG UNITS) = 63 COMBINING UNITS

FIGURE 1.

search laboratory in the hospital. In 37 of 40 determinations the titres obtained by the two technicians were identical and in the remaining 3 the differences were only one dilution. These results lend strong support to the belief that the changes observed are real and not due to inherent error in the technique.

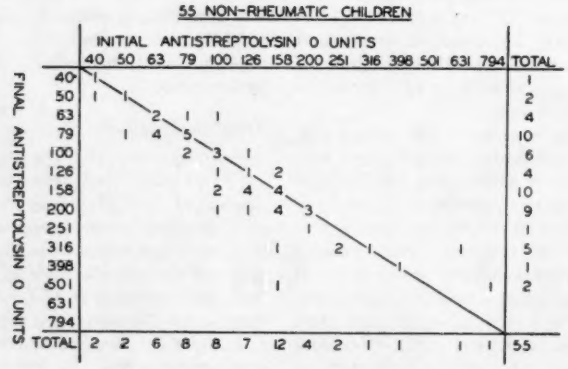
Antistreptokinase. The method of Kaplan and his associates (16) was employed but this test was not done in duplicate. The serum dilutions starting with 1:25 were based on a logarithmic scale in which each successive dilution was increased by a factor of 1.5 as described by Kaplan.

Antihyaluronidase. A modification (17) of the method of McLean (18) was used for this test. The serum was diluted in distilled water using the same scale as for antistreptokinase.

RESULTS

The initial geometric mean of antistreptolysin O titre for the 20 rheumatic children was 158

ANTISTREPTOLYSIN O TITERS OF BLOOD SERUM BEFORE AND 3 WEEKS AFTER INJECTION OF STREPTOLYSIN O CONCENTRATE



AVERAGE TITER BEFORE INJECTION = 2.08 ± .04 (LOG UNITS) = 121 COMBINING UNITS
AVERAGE TITER AFTER INJECTION = 2.13 ± .03 (LOG UNITS) = 135 COMBINING UNITS
AVERAGE RISE = 0.049 ± .017 (LOG UNITS) = 14 COMBINING UNITS

FIGURE 2.

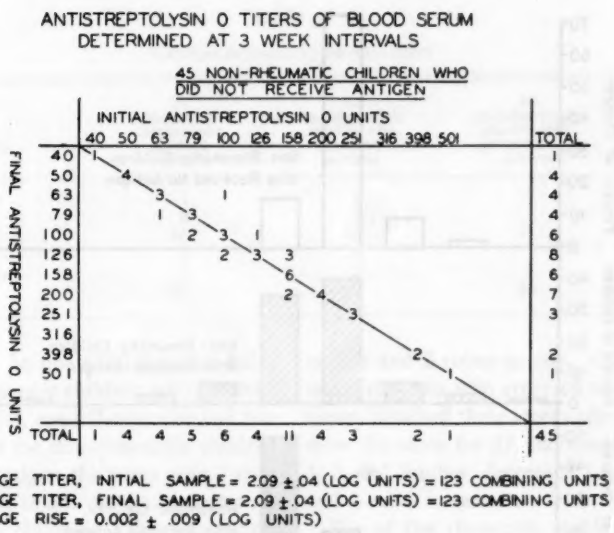

ANTISTREPTOLYSIN O TITERS OF BLOOD SERUM
DETERMINED AT 3 WEEK INTERVALS
45 NON-RHEUMATIC CHILDREN WHO
DID NOT RECEIVE ANTIGEN
INITIAL ANTISTREPTOLYSIN O UNITS
40 50 63 79 100 126 158 200 251 316 398 501
TOTAL
FINAL ANTISTREPTOLYSIN O UNITS
TOTAL 1 4 4 5 6 4 11 4 3 2 1 45
AVERAGE TITER, INITIAL SAMPLE = 2.09 ± .04 (LOG UNITS) = 123 COMBINING UNITS
AVERAGE TITER, FINAL SAMPLE = 2.09 ± .04 (LOG UNITS) = 123 COMBINING UNITS
AVERAGE RISE = 0.002 ± .009 (LOG UNITS)

FIGURE 3.

combining units before the administration of antigen and for the 100 non-rheumatic children it was 121 units. The mean titres, while higher in the rheumatic children, had a probability level of 0.07 of being a random sampling difference and therefore the differences were not statistically significant. The distribution of the initial antistreptolysin O titres for 20 rheumatic children is shown in Figure 1.

Three weeks after the injection of streptolysin O antigen, the levels for the rheumatic children had risen to a geometric mean titre of 221 combining units, a significant rise, although 5 of the children had shown no rise and only 5 had rises of two or more tubes in the dilution system. The antistreptolysin O level of the 55 non-rheumatic children who received antigen rose from 121 to a geometric mean titre of 135 combining units three weeks after the antigen was injected (Figure 2). This is also a significant rise though 29 of the 55 children showed no rise and only seven had a rise of 2 or more tubes. The greater average antistreptolysin O response of the rheumatic children to equivalent doses of streptolysin O concentrate was statistically significant ($P = 0.01$). The distribution of antistreptolysin O titres before and 3 weeks after injection of the streptolysin O concentrate is shown in Figure 2 for the 55 non-rheumatic children who received antigen. A group of 45 children who received no antigen was studied in the same manner as were the children who received antigen (Figure 3). Thirty-three of these children showed no change in antistreptolysin O titre during the three-week interval, seven had a 1-tube rise, four a 1-tube decrease and one a 2-tube decrease. The pre- and post-booster levels were 123 units. The percentage distribution of rise, no change, and decrease in antistreptolysin O titres shown in Figure 4 for rheumatic and non-rheumatic children who received antigen and the non-rheumatic children who did not receive antigen illustrates the tendency for the rheumatic children to respond with higher titres.

Correlation of the dose of antigen which each child received with the antistreptolysin O response indicates that the antistreptolysin O response is not a function of the quantity of antigen, at least not in this dosage range, because the children who received the larger doses, 50 to 80 units of streptolysin O, did not exhibit significantly higher antibody responses. Neither did the age of the children affect their response to streptolysin O concentrate significantly, because the titres of the children 11 or older were not significantly higher than the children 10 or younger, either in the rheumatic or non-rheumatic groups. The elapsed time since the onset of the most recent attack of rheumatic fever was not significantly dif-

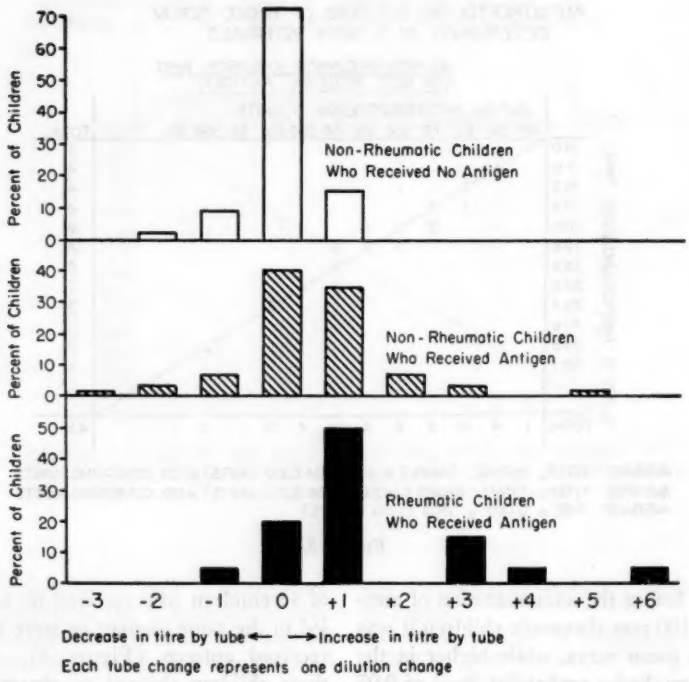

Percent of Children
Non-Rheumatic Children
Who Received No Antigen
Non-Rheumatic Children
Who Received Antigen
Rheumatic Children
Who Received Antigen
-3 -2 -1 0 +1 +2 +3 +4 +5 +6
Decrease in titre by tube ← → Increase in titre by tube
Each tube change represents one dilution change

FIGURE 4.

ferent for those rheumatic children with a 3-tube or greater response (20.1 months' average) when compared with the rheumatic children with less than a 3-tube rise (17.1 months' average) not including two children whose onset of rheumatic fever was five and one-half and eight years previously.

The possibility does exist that the rheumatic and non-rheumatic groups might not be strictly comparable because the rheumatics had been on prophylaxis and did not have a streptococcal infection recently whereas for the non-rheumatic children who received antigen, all that can be said is that they did not have an upper respiratory infection of any kind within 1 month prior to receiving antigen nor during the 3-week period of observation and that their throat cultures were negative for hemolytic streptococci before and 3 weeks after the antigen was administered.[2]

The geometric mean antistreptokinase titre of 159 units for 20 rheumatic children before antigen was administered was considerably higher than the mean of 148 units for 51 non-rheumatic children who received antigen and 100 units for 46 non-rheumatic children who received no antigen. However, a test for significant difference between mean titres for rheumatic and non-rheumatic children gives $P = 0.22$, clearly not statistically significant. The titres rose following antigen in only three rheumatic children, the highest rise being

[2] If a decreasing antistreptolysin O titre is used as evidence of past streptococcal infection, that is, one which occurred several weeks or months ago, in contrast to a rising titre as evidence of an infection within the past 2 to 3 weeks, the data indicate that 7 of the 55 non-rheumatic children who received antigen had a decrease in titre compared with 1 of 20 rheumatic children. If the antistreptolysin O changes of the three non-rheumatic children who had a decrease in titre of 2 or more tubes are eliminated, a t test comparing titre changes of 20 rheumatics with the remaining 52 non-rheumatics gives $P = 0.021$, still a significant difference. If all seven of the non-rheumatic children who had a decrease in titre of 1 tube or more are eliminated along with one rheumatic child who had a 1-tube decrease, comparison of titre changes gives $P = 0.021$, again a significant difference.

TABLE III

Changes in antistreptokinase titres

Change in antistreptokinase titres between initial and final samples	20 rheumatic children who received antigen	51 non-rheumatic children who received antigen	46 non-rheumatic children who received no antigen	Total
Two tubes lower	1	0	1	2
One tube lower	0	2	1	3
Same	16	44	41	101
One tube higher	2	4	2	8
Two tubes higher	1	1	1	3
Total	20	51	46	117

2 tubes (Table III). In one child the titre fell 2 tubes. Four non-rheumatic children who received antigen had a 1-tube rise, one a 2-tube rise and two a 1-tube decrease. In the non-rheumatic children who did not receive antigen the titres rose 1 tube in two children, 2 tubes in one child and decreased 1 and 2 tubes in two children. The small rises observed in only three rheumatic children and five non-rheumatic children who received antigen are not significantly different from the changes in titres observed in the children who did not receive antigen.

The "pre-booster" antihyaluronidase titres were distributed in a manner similar to those for antistreptolysin and antistreptokinase, *i.e.*, the mean initial levels were consistently higher for the rheumatic than the non-rheumatic children being 1 : 248, 1 : 174 and 1 : 177 for the three groups respectively. Three weeks following the streptolysin O concentrate, among the rheumatic children the titre remained the same in 17, rose 1 tube in one child and diminished 1 tube in two children (Table IV). Among 50 non-rheumatic children who received antigen, titres did not change in 38, rose 1 tube in five of them, 3 tubes in one and diminished 1 tube in five and 2 tubes in one. Of the 46 non-rheumatic children who received no antigen, titres for serum obtained three weeks after the first specimen were the same for 37, increased in three children, 1, 2, and 3 tubes, decreased 1 tube in five of them and 2 tubes in one.

Six of the rheumatic children and one of the non-rheumatics had severe reactions with local and systemic manifestations. These reactions were characterized by tenderness and edema gradually involving the entire arm, extensive erythema and increased temperature about the injection site and pain of the entire arm, all of these manifestations beginning 2 to 12 hours after the injection and increasing in area and severity for about 48 hours, thereafter decreasing. The systemic reaction began at the same time and consisted of fever lasting usually 3 days, but for 5 days in one child, reaching a maximum of 102 to 105°F., generalized malaise, anorexia, vomiting in one child and pain and tenderness in an interphalangeal joint of the hand opposite the injection side in one child. The remainder of the rheumatic and non-rheumatic children who received antigen had either no noticeable reaction or mild to moderate tenderness and

TABLE IV

Changes in antihyaluronidase titres

Change in antihyaluronidase titres between initial and final samples	20 rheumatic children who received antigen	50 non-rheumatic children who received antigen	46 non-rheumatic children who received no antigen	Total
Two tubes lower	0	1	1	2
One tube lower	2	5	5	12
No change	17	38	37	92
One tube higher	1	5	1	7
Two tubes higher	0	0	1	1
Three tubes higher	0	1	1	2
Total	20	50	46	116

erythema about the injection site. The occurrence of a severe reaction in six of the rheumatic children and only one non-rheumatic would seem to be of significance; at any rate because of these severe reactions the decision was made not to give the antigen to any more rheumatic children. There was no clinical or laboratory evidence of reactivation of rheumatic fever in any of the inactive rheumatics, exacerbation of rheumatic fever in any of the active rheumatics, or initiation of rheumatic fever in any of the non-rheumatics. The C-reactive protein reaction did not become positive in any rheumatic child after antigen administration. Three of these children received 40 units and three 60 units of antigen; one had a 6-tube rise, five a 1-tube rise and one had no rise in antistreptolysin O.

DISCUSSION

The finding that the children with rheumatic fever responded as a group with significantly higher antistreptolysin O titre rises than non-rheumatic children following equivalent doses of streptolysin O would be of greater significance if all the rheumatic children and none of the non-rheumatics had reacted in this manner. If rheumatic subjects are all fundamentally different in respect to the manner in which they respond to streptococcal infections or antigens, it might be expected that they would all exhibit higher antibody rises. An explanation of these findings does not necessarily lie in any inherent difference in the manner in which rheumatic children respond to streptococcal antigens. The individual's past experience with the hemolytic streptococcus, that is, the total number and severity of infections and the elapsed time since the most recent infection certainly determines in part the magnitude of clinical and antibody response. However, only 5 of the 20 rheumatic children studied responded with large rises in titre and if these five are eliminated there would have been no significant differences between the rheumatics and non-rheumatics.

The absence in all but two children of significant rises in antistreptokinase titres following the injection of an antigen containing a very small amount of streptokinase suggests the amount of antigen may have been too small to stimulate an antibody response. The work of Christensen (20) and Tillet and Sherry (21) leaves no doubt that streptokinase is antigenic. Antihyaluronidase titres rose significantly in only two children who received antigen. The failure to demonstrate consistent rises in either antistreptokinase or antihyaluronidase levels during the period of observation is evidence that non-specific and anamnestic antibody responses did not occur and is in accord with the observations of Kuhns and McCarty (10) that non-specific antibody responses do not occur.

The changes in antistreptolysin O titres which took place in the non-rheumatic children who did not receive streptolysin O indicate that antistreptolysin O levels are continually changing in some children. Whether the titre is rising or falling or remaining stationary probably depends on how recently the child has had a streptococcal infection. The marked changes which occur in this and other streptococcal antibodies in normal children over a period of time has been observed previously (22, 23).

The streptolysin O concentrate evoked a marked systemic reaction in only 1 of 55 non-rheumatic children whereas 6 of 20 rheumatic children reacted severely to an equivalent dose. This striking difference indicates that some rheumatic children, as least, react systemically as well as in their antibody response in a manner significantly different from non-rheumatics; however, only one of these six had an ununusally high antistreptolysin O response. The meaning of this difference is not clear nor is the cause, but one cannot help wondering if the experience of these few children with the hemolytic streptococcus either in the recent or distant past might have been different from the others. That is, if these seven children who had severe reactions had unusually numerous streptococcal infections, would they be more likely to have a more severe systemic reaction or respond with higher antibody levels than those who had fewer or more distant infections? The elapsed time since the most recent attack of rheumatic fever and presumably the last streptococcal infection was not significantly different for the "severe reactions," and time *per se* did not seem to determine which of the rheumatic children would react most severely or would exhibit the highest antibody responses.

The findings of this study are similar to others which have attempted to show a direct relation-

ship between rheumatic fever and streptococcal infections; the results although suggestive are not conclusive. Some of these rheumatic children reacted differently to the streptolysin antigen, but some did not. Possibly susceptibility to rheumatic fever is a changing process similar to immunity against certain infections and if such antigen-response measurements could be made at a time when the rheumatic children are highly susceptible (whenever that might be) clear-cut, conclusive differences might be shown.

SUMMARY

Streptolysin O concentrate was prepared and administered intramuscularly to 20 children with rheumatic fever and 55 non-rheumatic children. Another group of 45 non-rheumatic children served as an additional control. The children were carefully observed clinically and antistreptolysin O, antistreptokinase and antihyaluronidase titres were determined before and 3 weeks after administration of the antigen.

The initial geometric mean antistreptolysin O titre of 158 combining units for the rheumatic children was higher than the initial mean titre of 121 for 100 non-rheumatic children; this difference is not statistically significant.

Initial antistreptokinase and antihyaluronidase titres were higher for the rheumatic children than the non-rheumatic but the differences were not great. No significant rise in either of these mean antibody levels following antigen occurred, indicating that non-specific or anamnestic antibody responses did not occur following streptolysin O administration. Six rheumatic and one non-rheumatic child had rather severe local and generalized reactions to the antigen. No evidence of initiation or reactivation of rheumatic fever was observed.

The results indicate that, as a group, the children with rheumatic fever reacted more severely and had greater antistreptolysin O responses following the intramuscular injection of streptolysin O concentrate than the non-rheumatic children. The significance or reasons for the findings are not clear, but the results do support previous clinical observations that rheumatic children frequently but not always respond with higher antibody levels following a streptococcal infection than non-rheumatic children.

ACKNOWLEDGMENT

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THE CONCENTRATION AND BINDING OF THYROXINE AND TRIIODOTHYRONINE BY RAT DIAPHRAGM [1]

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Thyroid hormone has long been thought to exert its primary effect in the peripheral tissues. Recent studies have indicated, however, that thyroxine may be converted to other products in these tissues before becoming metabolically active (1–4). Whether or not a subsequent alteration is necessary, it seems probable that the thyroxine must first be fixed in some form of physicochemical union, either on the surface of, or inside the cell, before it can exert its characteristic effect on the metabolism of the tissue.

Probably the most complete study of this sort has been carried out by Stadie on the binding *in vitro* of insulin to various rat tissues, particularly diaphragm (5, 6). With respect to thyroxine and triiodothyronine, Hamolsky and Crispell and coworkers have studied the effect of plasma on the binding of these hormones to red blood cells *in vitro* (7–9) and Gross has reported similar studies relative to liver slices (10).

Since one significant site of thyroid hormone localization seems to be the muscle mass of the body (11), it should be of interest to study the binding *in vitro* of thyroxine to a specific tissue such as rat diaphragm. In fact, one such study has been reported by Hamolsky and Freedberg (12) who investigated differences in the rate of uptake by rat diaphragm of thyroid hormone from the plasma of normals and thyrotoxic patients. The purpose of this report is to present information on the nature of the binding phenomenon exhibited by rat diaphrgam *in vitro*, relative to thyroxine and triiodothyronine labeled with I^{131}.

MATERIALS AND METHODS

Hemidiaphragms from 200-Gm. male Sprague-Dawley rats were used throughout the experiment. All rats were bred in our labortory and maintained at a constant room temperature on Purina laboratory chow.

Diaphragms were removed from the rats after ether anesthesia, bisected, weighed and immediately immersed in 5 ml. of phosphate buffer (5) containing varying amounts of I^{131}-labeled l-thyroxine or l-triiodothyronine [2] and incubated at 37°C. in a Dubnoff shaker for varying periods of time according to the conditions of the experiments. After removal from the incubating beakers the hemidiaphragms were rinsed three times in 25 ml. of ice cold phosphate buffer. The first two rinses were always of 30 seconds' duration and the time of the third rinse varied as indicated in each experiment. After the third rinse, the tissue was dissolved in a constant volume of 30 per cent KOH and the radioactivity in the solution determined with a well-type gamma counter. Results are expressed in terms of micromoles bound per 100 mg. of diaphragm as determined by calculation from the specific activity of the labeled compounds used. Comparison studies, using NaI^{131}, were carried out under similar conditions.

All points in all experiments reported represent the average values for 12 hemidiaphragms. The range of values was wide in some instances (see Figure 1) and it was found necessary to use this number of hemidiaphragms to obtain statistically significant results. Both hemidiaphragms from the same animal were never used in the determination of a single point.

EXPERIMENTAL CONDITIONS AND RESULTS

Incubation time

In order to determine the effect of increasing time of incubation on the binding of thyroxine by rat muscle, hemidiaphragms were incubated with concentrations of I^{131}-labeled thyroxine ranging from 1.3×10^{-4} through 26.0×10^{-4} μM per ml. for intervals of 1, 5, 15, 30, 60, 120, or 240 minutes. The third rinse was of 30 minutes' duration in all cases.

Figure 1 shows the results of this study when

[1] Supported in part by grants from the U. S. Atomic Energy Commission and the U. S. Public Health Service.

[2] Obtained from Abbott Laboratories, Oak Ridge, Tennessee. Both hormones were labeled in the position ortho to the hydroxyl group.

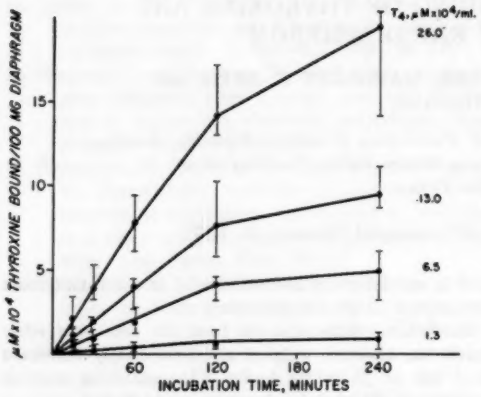

FIG. 1. BINDING OF THYROXINE-I^{131} TO RAT DIAPHRAGM WITH INCREASING INCUBATION TIME

Concentration of thyroxine (T4) in incubation medium is shown at the right. In this and all subsequent figures, concentrations along ordinates or when listed in a column are multiplied by 10^4 to give unit values, *i.e.*, $26 \times 10^{-4} \times 10^4 = 26$. Hence ordinates are labeled $\mu M \times 10^4$ indicating multiplication by this factor. Ranges of values are indicated by the thin vertical line at each point and are presented as an illustrative example in Figure 1 only.

the amount of thyroxine-I^{131} fixed to tissue is plotted against time. The figures at the end of each curve indicate the hormone concentration in the incubation medium. It can be seen that at each concentration the amount of thyroxine bound was linearly related to time for approximately 120 minutes: there appeared to be some decrease in the rate of uptake by 240 minutes. Recalculation of these figures in relative terms (Table I) shows that the per cent of available thyroxine-I^{131} bound at any given time was independent of its concentration in the medium over the 240-minute period studied.

It is of some significance to point out that, regardless of the initial concentration of the hormone and despite rinsing for over 30 minutes, the concentration of thyroxine bound to diaphragm very closely approximated that in the medium within 15 minutes and clearly exceeded this amount by 30 minutes.

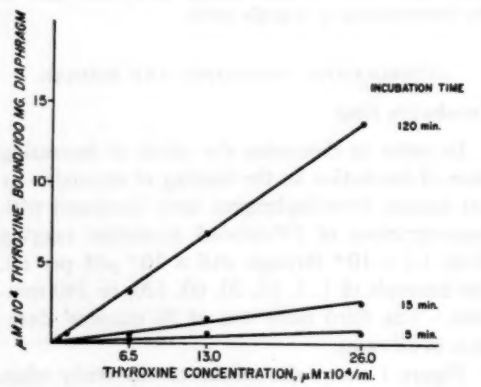

FIG. 2. BINDING OF THYROXINE-I^{131} TO RAT DIAPHRAGM AS A FUNCTION OF THYROXINE CONCENTRATION IN INCUBATING MEDIUM

Incubation time is shown at right.

The role of thyroxine concentration

The above experiments were repeated and the results are presented in Figure 2, wherein the concentration of thyroxine-I^{131} initially present in the incubation medium is plotted against the amount of hormone bound per 100 mg. of rat hemidiaphragm after incubation for 5, 15 and 120 minutes. It should be noted that these intervals represent times prior to, and subsequent to, the achievement of tissue concentrations of the hormone greater than those initially present in the medium (Figure 1). It may be seen that, regardless of these intervals, there was a direct linear relationship between the amount of thyroxine available for binding and amount that was bound by the hemidiaphragms. Consequently, these experiments show that for concentrations up to 26×10^{-4} μM per ml. and for incubation periods up to 120 minutes, no limit of the concentrating and binding ability of the tissue had been reached.

TABLE I

Per cent of available thyroxine bound to rat diaphragm with increasing incubation time *

Incubation time *Minutes*	Per cent available thyroxine bound per 100 mg. diaphragm
15	0.7 (0.6–0.8)
30	2.7 (2.2–3.5)
60	6.0 (5.7–6.3)
120	10.5 (8.0–12.9)
240	14.6 (13.5–15.4)

* Thyroxine concentration ranged from 1.3×10^{-4} μM per ml. through 26.0×10^{-4} μM per ml.

The relationship between rinsing time and thyroxine-I^{131} binding

To determine the rate at which radioactivity can be removed from diaphragm after incubation with thyroxine-I^{131}, and hence to determine the degree of binding to the tissue, the following experiment was designed. Hemidiaphragms were incubated in buffer containing 1.3×10^{-4}, 6.5×10^{-4} or 13.0×40^{-4} μM of thyroxine-I^{131} per ml. for 1, 15 or 120 minutes. They were then rinsed as described previously except the third rinse lasted 1, 5, 15, 30 or 45 minutes. Results of studies, where incubation was carried out for 1 minute and 120 minutes are shown in Figures 3 and 4. These curves are strikingly similar to those obtained by Stadie, Haugaard, and Vaughen (6) for insulin-I^{131} under similar conditions and indicate that little thyroxine-I^{131} is removed from muscle after rinsing for approximately 15 minutes. Furthermore, these results support the view that, of the total thyroxine associated with the tissue, the amount firmly bound is proportional to the concentration in the medium.

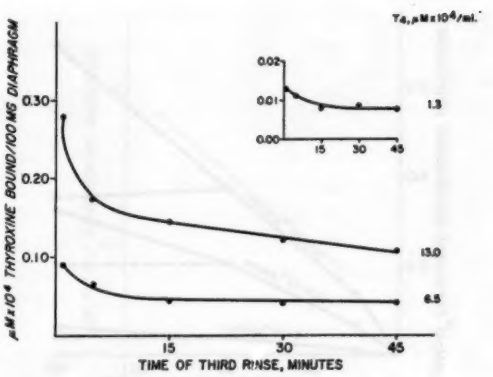

FIG. 3. AMOUNT OF THYROXINE-I^{131} BOUND TO RAT DIAPHRAGM AFTER INCUBATION FOR ONE MINUTE AND RINSING FOR INCREASING PERIODS OF TIME

Concentration of thyroxine (T4) in incubating medium is shown at right.

Equilibrium studies

In a further attempt to elucidate the nature of the binding of thyroxine to muscle tissue, equilibrium studies were carried out using non-labeled thyroxine. Hemidiaphragms were incubated in buffer containing 1.3×10^{-4}, 6.5×10^{-4} or 13.0×10^{-4} μM of thyroxine-I^{131} per ml. for up to 120 minutes. The length of the third rinse was 30 minutes in all instances.

For each concentration, 12 hemidiaphragms were removed at the 15-minute point and assayed for radioactivity. At 60 minutes a second set of 12 hemidiaphragms was removed and assayed while a third set was rinsed twice for 30 seconds and transferred to an identical solution containing non-radioactive thyroxine. After an additional 60 minutes' incubation in this solution, the hemidiaphragms were rinsed for 30 minutes in buffer and the radioactivity determined as before. A fourth set was incubated in the radioactive thyroxine solution for the full 120 minutes. The results are presented in Figure 5.

The results of this equilibrium study show that little or none of the thyroxine-I^{131} bound to muscle during the first 60 minutes of incubation was lost by incubation with unlabeled thyroxine for another equal period of time at any of the concentrations used. This study confirms the view that the concentration of thyroxine by muscle involves a firm binding rather than the gradual approximation of an equilibrium state. It would appear probable that once a molecule of thyroxine is fixed to tissue it is not released as such but rather as some conversion product.

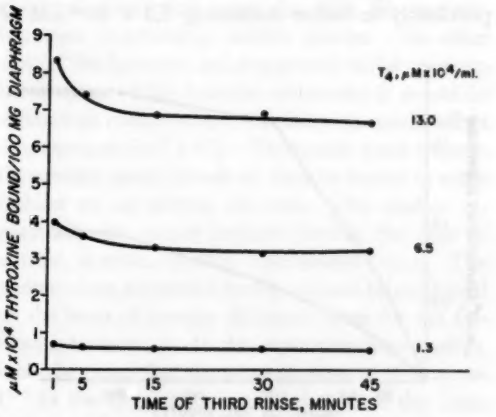

FIG. 4. AMOUNT OF THYROXINE-I^{131} BOUND TO RAT DIAPHRAGM AFTER INCUBATION FOR 120 MINUTES AND RINSING FOR INCREASING PERIODS OF TIME

Concentration of thyroxine (T4) in incubating medium is shown at right.

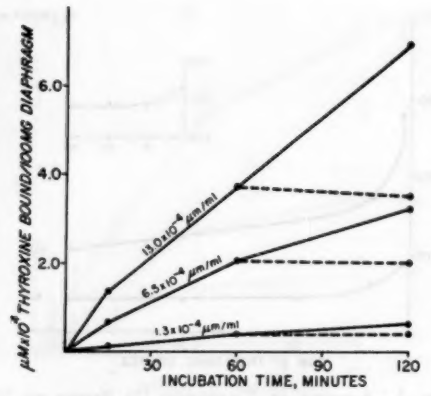

FIG. 5. EQUILIBRIUM STUDY—THYROXINE-I^{131} BOUND TO RAT DIAPHRAGM WITH INCREASING INCUBATION TIME

At the 60-minute point diaphragms were removed and incubated in non-labeled thyroxine for an additional 60 minutes. Solid line indicates thyroxine-I^{131} in incubation medium; broken line indicates transfer to unlabeled thyroxine.

Binding and washout of triiodothyronine

Since triiodothyronine has been shown to act much more rapidly than thyroxine in raising the oxygen consumption of both animals and man (13–16) it seemed of interest to determine whether the binding of this compound by rat muscle differed from that of thyroxine.

Hemidiaphragms were incubated as described previously in buffer containing 1.3×10^{-4} μM of triiodothyronine-I^{131} per ml. for periods of 1, 5, 15, 30, 60, 120 or 240 minutes and were rinsed 3 times, the third rinse lasting 30 minutes. The values obtained are presented in Figure 6 along with those from an identical experiment using thyroxine-I^{131}.

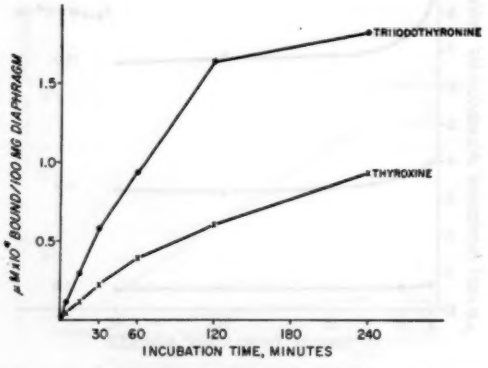

FIG. 6. COMPARISON OF RATE OF BINDING OF THYROXINE-I^{131} AND TRIIODOTHYRONINE-I^{131} TO RAT DIAPHRAGM

Hormone concentration in the incubation medium was 1.3×10^{-4} μM per ml.

It is evident that triiodothyronine-I^{131} is much more rapidly bound to rat diaphragm than is thyroxine-I^{131}. Furthermore, with one exception and regardless of the incubation interval, the ratio (triiodothyronine bound/ thyroxine bound) is quite constant, varying from 2.4 to 2.7; the exception occurs at 240 minutes, the ratio dropping to slightly less than 2. Various explanations for this drop may be offered; it is significant, however, that by 240 minutes almost 30 per cent of the available hormone had been firmly bound and, reasoning from the data in Figure 3, probably another 30 per cent was loosely entrapped in the tissue.

Washout studies similar to those carried out with thyroxine-I^{131} were performed with triiodothyronine-I^{131}. Hemidiaphragms were incubated for 1, 5 and 15 minutes in buffer containing 1.3×10^{-4} μM per ml. of triiodothyronine-I^{131}. The third rinse was carried out for 15, 30 and 45 min-

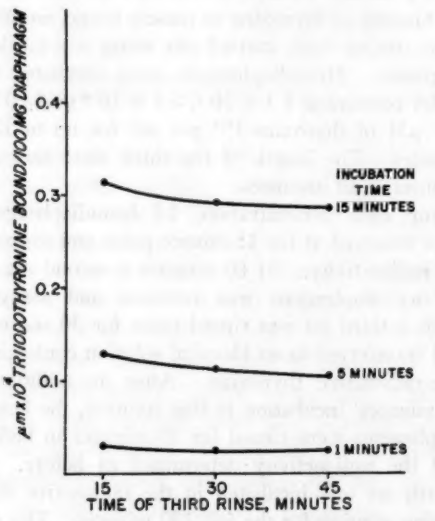

FIG. 7. AMOUNT OF TRIIODOTHYRONINE BOUND TO RAT DIAPHRAGM AFTER INCUBATION FOR 1, 5 AND 15 MINUTES AND RINSING FOR INCREASING PERIODS OF TIME

Concentration of triiodothyronine was 1.3×10^{-4} μM per ml.

utes. The results are presented in Figure 7. Comparison with Figures 3 and 4 shows the washout curves for triiodothyronine-I^{131} to be almost exactly parallel to those for thyroxine-I^{131}. The concentration of triiodothyronine-I^{131} in the tissue was consistently higher than that shown in the thyroxine-I^{131} concentration studies.

NaI^{131} studies

It is important to establish, in relative terms, to what extent iodide-I^{131} ion might influence the results presented in previous sections. Consequently, a study was conducted in which hemidiaphragms were incubated for various periods of time up to 240 minutes in buffer containing 6.5×10^{-4} μM per ml. of sodium iodide-I^{131}. The third rinse was for 30 minutes. Figure 8 presents the results for this experiment; the curve for a similar experiment involving an equimolar concentration of thyroxine-I^{131} is presented for comparison. The amount of NaI^{131} bound to muscle was clearly different from the amount of thyroxine-I^{131} bound and was, in quantitative terms, insignificant.

Washout studies similar to the experiment represented by Figures 3 and 4 produced the results shown in Figure 9. In the instance shown, the diaphragms were incubated for 120 minutes in medium containing 6.5×10^{-4} μM NaI^{131} per ml. and the third wash was of 15, 30 and 45 minutes' duration. This study indicated a rapid and continued loss of NaI^{131} from tissue as the rinsing period was prolonged; furthermore, the slope of this curve differed from those for thyroxine-I^{131} and triiodothyronine-I^{131}.

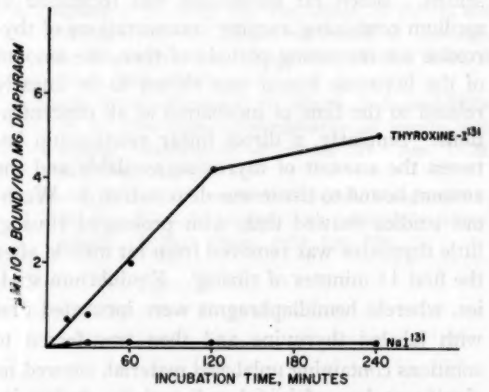

FIG. 8. COMPARISON OF RATE AND AMOUNT OF BINDING OF THYROXINE-I^{131} AND NaI^{131} TO RAT DIAPHRAGM

Concentration of both compounds was 1.3×10^{-4} μM per ml.

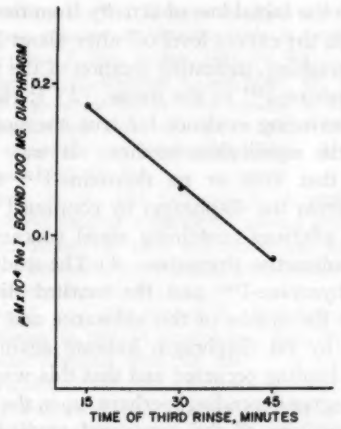

FIG. 9. AMOUNT OF NaI^{131} REMAINING IN RAT DIAPHRAGM WITH RINSING FOR INCREASING PERIODS OF TIME

Diaphragms were first incubated for 120 minutes in medium containing 6.5×10^{-4} μM NaI^{131} per ml.

DISCUSSION

As pointed out by Stadie it would seem reasonable that the Michaelis-Menten concept of enzyme-substrate interaction must also hold for enzyme-hormone relationship within tissues. In other words "the hormone must approach within molecular distance of the enzyme, otherwise it would be difficult to conceive how the hormone could affect enzymatic action" (17). Thyroxine (and triiodothyronine) must, therefore, first be bound in some fashion to, or within, the cell. The studies reported in this paper indicate that in the case of muscle, *in vitro*, binding does indeed occur. The observations presented here could not be explained on the basis of passive diffusion alone for the following reasons: 1) In the incubation time studies, it can be noted that the concentration of thyroxine-I^{131} in the diaphragm exceeded that of the incubating medium after an incubation period of about 15 to 20 minutes. Since no corrections for tissue water are taken into account here, the true concentrations in the tissue must have been even higher. 2) The washout studies showed that,

although the initial loss of activity from the tissues was high, the curves level off after about 15 minutes of washing, indicating fixation of the remaining thyroxine-I^{131} to the tissue. 3) Perhaps the most convincing evidence for true chemical binding is the equilibrium studies. It was demonstrated that little or no thyroxine-I^{131} was removed from the diaphragm by continued incubation in solutions containing equal concentrations of nonradioactive thyroxine. 4) The studies with triiodothyronine-I^{131} and the marked difference between the uptake of this substance and thyroxine-I^{131} by rat diaphragm indicate again that a specific binding occurred and that this was different in degree depending, perhaps, upon the physiological activity of the compound studied. Certainly, if only passive diffusion into the tissue was involved, one might expect little difference between these chemically similar compounds. The uptake and washout studies with NaI^{131}, where binding by the tissue was far different in degree from that observed with thyroid hormone, lend further support to the concept of specific binding of thyroxine and triiodothyronine.

It is of some interest to note that binding of these hormones to tissue is probably not the sole mechanism for their removal from the incubation medium. The two preliminary rinses for 30 seconds each removed the major portion of the labeled hormone that was held in the surface film of fluid on the diaphragm, yet it took more than 15 minutes to remove what appeared to be unbound thyroxine (Figures 3 and 4) from the tissue. In view of the fact that the initial values in these figures were also proportional to both the incubation time and concentration of hormone in the medium, it seems only reasonable to suggest that both thyroxine and triiodothyronine were actively transported from the medium into the cell where binding to cellular loci took place resulting in the firm fixation reported in these experiments. The remaining unbound hormone within the cell was available for diffusion back into the medium. Compartmentalization of this type within the cell has previously been reported in rat liver (18).

The observation that triiodothyronine is much more rapidly bound to muscle tissue than is thyroxine corresponds well with observations in man that this substance acts four to five times as rapidly as thyroxine in raising the oxygen consumption of intact animals. After 120 minutes, for example, almost three times as much triiodothyronine-I^{131} was incorporated as was thyroxine-I^{131}. However, since both substances are bound to the tissue this experiment neither supports nor contradicts the hypothesis that thyroxine must be converted to triiodothyronine before becoming physiologically active.

Finally, it would seem that since thyroxine and triiodothyronine are specifically bound to rat diaphragm, this observation may offer a convenient means of studying the effects of varying *in vivo* and *in vitro* conditions on the peripheral action of these compounds. Furthermore, the rate of active transport and binding of these hormones may be a factor of paramount significance in understanding the mechanism of their action. In this regard, it has been observed that an increased rate of peripheral degradation of thyroxine-I^{131} was coincident with increased thyroidal activity in clinically euthyroid relatives of patients with Graves' disease (19). The role of active transport and binding of thyroid hormone may well be one of the regulatory factors in maintaining the euthyroid state.

SUMMARY

Studies of the uptake of I^{131}-labeled thyroxine by rat diaphragm as a function of incubation time, hormone concentration in the medium and duration of rinsing, as well as equilibrium studies, are presented. When rat diaphragm was incubated in medium containing varying concentrations of thyroxine for increasing periods of time, the amount of the hormone bound was shown to be linearly related to the time of incubation at all concentrations. Similarly, a direct linear relationship between the amount of thyroxine available and the amount bound to tissue was demonstrated. Washout studies showed that, with prolonged rinsing, little thyroxine was removed from rat muscle after the first 15 minutes of rinsing. Equilibrium studies, wherein hemidiaphragms were incubated first with labeled thyroxine and then transferred to solutions containing unlabeled material, showed no significant loss of initial radioactivity during incubation with the non-radioactive thyroxine. All of these observations indicate that thyroxine is indeed specifically bound to rat muscle tissue in

concentrations far exceeding those in the incubating medium.

Similar incubation and washout studies were carried out with triiodothyronine. These studies showed not only that triiodothyronine was also specifically bound to rat muscle tissue but that this binding took place much more rapidly than in the case of thyroxine. When the hormone concentration was 1.3×10^{-4} μM per ml. triiodothyronine-I^{131} was bound to rat diaphragm 2.5 times as rapidly as was thyroxine-I^{131}. This finding is in accord with previous observations regarding the relative rates of action of these two compounds *in vivo*.

Comparison studies carried out with NaI^{131} showed that, in equimolar concentrations, relatively insignificant amounts of this compound were taken up by rat diaphragm and that which was absorbed was readily removed by washing.

These investigations are discussed in relation to the mechanism of action of thyroxine and triiodothyronine.

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UNESTERIFIED FATTY ACID IN HUMAN BLOOD PLASMA. II. THE TRANSPORT FUNCTION OF UNESTERIFIED FATTY ACID

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In a recent publication (1), studies of the concentration of unesterified fatty acid (UFA) in the blood of normal human subjects were reported. The relation of the concentration of unesterified fatty acid to the nutritional state of the subject, together with the observation of arteriovenous differences in unesterified fatty acid concentration, formed the basis for the belief that this lipid fraction constitutes a transport form of fatty acid which can account for the movement of fats from adipose tissue depots into tissues requiring fat as an energy-yielding substrate. Other studies were reported which served to indicate the existence of hormonal mechanisms controlling the concentration of circulating unesterified fatty acid. At the same time, there appeared another publication (2), representing independent investigations with a different analytical method, in which virtually identical observations were reported (with the exception that no studies of arteriovenous differences were included). In view of the potentially great physiologic significance of plasma unesterified fatty acid, it was decided that the above-mentioned studies should be confirmed and extended, and that another analytical procedure, more accurate and convenient than that used earlier, should be developed. The method employed by Dole (2), though extremely convenient and rapid, did not seem suitable for the investigation of arteriovenous differences, since lactate interferes appreciably with the results obtained by that method. The data reported in the present publication were obtained by a procedure which has the advantage of high specificity, although it is appreciably more laborious than the method of Dole.

MATERIALS AND METHODS

Two groups of hospitalized patients were utilized as subjects for this study. The observations on arteriovenous differences were made on patients undergoing cardiac catheterization for the diagnosis of organic heart disease. These subjects were not strictly normal, but they had all received medical treatment as indicated to alleviate their symptoms, and none was used for this work who had clinically demonstrable congestive failure or suffered from any intercurrent metabolic disorder. They were fasting when studied, and were at rest physically, though for the most part there was considerable apprehension concerning the procedure to be carried out. The observations involving sequential samples of blood from the antecubital vein were made on normal conscientious objectors. These young men were hospitalized, had had a complete medical investigation, and were free of disease. They were fasting when studied, at rest physically, and, in general, were not greatly concerned emotionally with the procedure. In the observations involving sequential sampling of venous blood, care was taken to obtain all samples of a series from the same point in the same vein, in order to avoid superposing on the time course of the fatty acid concentration variations related to the fact that different veins might drain tissues whose behavior in extracting unesterified fatty acid from, or adding it to, blood might not be identical. Ideally, such studies should be done on arterial blood samples, but the advantages of arterial sampling were felt not to justify the discomfort involved.

Blood for analysis was collected in Wintrobe's oxalate mixture, refrigerated promptly, and centrifuged in the cold. An aliquot of plasma (1 to 3 ml., depending on the expected UFA concentration) was lyophilized in a 40-ml. conical glass-stoppered centrifuge tube, and the dry powder extracted for 30 minutes or more with exactly 25 ml. of a mixture of equal volumes of glacial acetic acid (Reagent Grade) and isoöctane (2,2,4 trimethyl pentane, Pure Grade, 99 per cent mol fraction minimum, obtained from Phillips Petroleum Co., Bartlesville, Oklahoma). Neither solvent required redistillation or other purification prior to use. The mixture was most conveniently delivered into the extraction vessels with a Krogh pipette. The tubes were then centrifuged, and the solvent decanted into a 60-ml. separatory funnel containing 2.5 ml. of 0.05 per cent sulfuric acid. After being shaken thoroughly, the funnel was centrifuged at approximately 100 G for 5 minutes to effect complete separation of the two phases. The lower phase was discarded, and the upper octane phase washed twice with 25 ml. of the same dilute sulfuric acid mixture, with recentrifugation after each washing and again after the second washing had been drained from the funnel. Ordinary

glass separatory funnels with the stems cut off at 1 cm. have proven satisfactory. The stopcocks may be greased with a product whose acid-base equivalence is negligible; for this purpose Lubriseal® stopcock grease has been employed. The stopcock cores may be held firmly in place by a rubber band looped from the body of the funnel, over the handle of the stopcock core, and back to the stem. With these precautions, the funnels withstand gentle centrifugation without leakage, provided there is always an aqueous layer between the octane and the stopcock. After completion of the washing steps, two 5-ml. aliquots of the octane solution were pipetted into 12-ml. conical glass centrifuge tubes, 1 ml. of Nile Blue indicator solution was added, and the latter titrated to a pink end point with 0.02 normal aqueous sodium hydroxide solution delivered from a suitable microburette. Nitrogen gas served simultaneously to mix the two phases and to flush away atmospheric carbon dioxide. The Nile Blue indicator solution was freshly prepared by mixing nine parts of absolute ethanol and one part of a stock aqueous solution of commercial Nile Blue A (0.02 per cent in water, washed with octane until free of extractable colored impurities). The titration was performed in duplicate on every extract, and the results averaged. After deduction of a reagent blank, this figure was used to calculate a single UFA concentration. Where duplicate values are reported, two identical plasma aliquots were lyophilized and four titrations carried out.

Statistical analysis of over one hundred plasmas analyzed in duplicate for unesterified fatty acid content indicated the standard error for a single determination to be 0.02 mEq. per L. Recovery experiments with stearic, palmitic, and oleic acids[1] added to plasma in the concentration of 1 mEq. per L. indicated 96 to 102 per cent recovery. Many carboxylic acids, anticoagulants, and other substances were added to plasma *in vitro* without producing an appreciable difference in the measured UFA concentration, whereas a recovery of 0.2 per cent of most of the added materials should have been noticeable. The substances so tested included acetoacetate, beta hydroxybutyrate, succinate, pyruvate, lactate, urate, cholate, desoxycholate, oxalate, ethylenediaminetetraacetate, heparin, and protamine. Only in the case of lithocholate was a small recovery of the added material observed, amounting to 3 per cent of the lithocholate introduced. A number of the washed octane extracts have been tested for the presence of lipid phosphorus; the average concentration found amounted to 0.003 mg. per ml. of plasma extracted. This amount of phospholipid is felt to be too small to introduce an appreciable error into the titration.

The analyses were for the most part begun within four hours after the withdrawal of the samples. When necessary, however, samples have been stored for periods ranging from overnight to several weeks prior to analysis. Experiment has shown that the samples may be stored without measurable increase in the UFA concentration at any one of three stages of the process, either as frozen plasma at − 20° C., after lyophilization and addition of the extracting mixture (stored stoppered at 4° C. to prevent evaporation of the organic solvents), or after the extracts have been washed and aliquots pipetted out for titration. Only deep-frozen plasma has been stored for more than 5 days. The result of the UFA determination on a plasma sample stored in the liquid state at 4° C. or as lyophilized powder exposed to air was found not to be reliable.

[1] These acids were obtained from the Hormel Institute, Austin, Minnesota.

TABLE I

A-V differences in UFA concentration from coronary sinus (C.S.) and hepatic vein (H.V.) samples *

Patient	Venous site	UFA (*mEq./L.*) Arterial	Venous	Diff.	O_2 extraction ratio %
M. C.	C. S.	.96	.64	.32	87
K. M.	C. S.	.77	.53	.24	70
F. E.	C. S.	1.04	.73	.31	70
G. P.	C. S.	.37	.21	.16	38
L. McQ.	C. S.	.82	.71	.11	36
N. C.	H. V.	.29	.24	.05	31
F. C.	H. V.	.94	.74	.20	103
A. P.	H. V.	1.02	.73	.29	
M. Q.	H. V.	1.03	.74	.29	
J. S.	H. V.	.59	.42	.17	

* The figures reported are means of duplicate analyses. Oxygen extraction ratio is obtained by dividing the amount of oxygen required for the complete combustion of the extracted UFA by the total oxygen extraction measured on the same blood samples. In the calculation of oxygen required, it is assumed that the fatty acids are of average molecular weight 275, and that no extractable UFA is carried in the erythrocytes.

OBSERVATIONS

To the data reported previously (1), there are now to be added a considerable number of new determinations of arteriovenous differences. The extraction of UFA from the blood perfusing myocardium and that perfusing the enteric circulation in fasting subjects has been confirmed with the use of the new analytical procedure. New data on coronary sinus and hepatic vein A-V differences are listed in Table I. In addition to these sites, blood samples have been obtained from the greater saphenous vein immediately below its junction with the femoral vein, the vein having been exposed at this location for the introduction of a cardiac catheter. Large negative A-V differences were encountered (Table II). Through the courtesy of Drs. Reuben Andres and Kenneth Zierler, twenty-two pairs of arterial and venous blood samples from the forearm muscles of fasting normal human subjects were made available. These samples were obtained by the techniques

TABLE II

A-V differences in UFA concentration from greater saphenous vein samples taken at cardiac catheterization *

Patient	UFA (*mEq./L.*)		
	Arterial	Venous	Diff.
W. E.	.50	.84	−.34
M. H.	.65	.77	−.12
E. D.	.63	1.50	−.87
C. R.	.84	1.80	−.96
J. W.	.87	1.64	−.77

* The figures are means of duplicate analyses.

recently described (3), and were transported in the frozen state to this laboratory for analysis. In fifteen of these twenty-two pairs, there was found a positive arteriovenous difference in the UFA concentration which exceeded twice the standard error of the analytic procedure. Four of the remaining paired samples showed an equally significant negative difference, and the remainder were inconclusive. These data will be reported later in more detail and correlation with other arteriovenous differences. Finally, an attempt has been made to demonstrate extraction of UFA from blood perfusing the kidney *in vivo*. Suitable samples were obtained in nine patients undergoing cardiac catheterization. The arteriovenous differences ranged from + 0.08 to − 0.03 mEq. per L., with both mean and median values of + 0.01, a difference which is unfortunately small in comparison with the experimental error.

There have been a number of instances in which it has been possible to administer glucose and insulin intravenously to a subject while obtaining sequential samples for the measurement of an arteriovenous difference of UFA concentration. One such example was reported previously (1), the venous site being the coronary sinus. Similar studies have since been carried out in three patients with catheters remaining in the hepatic vein, and in three in whom serial samples were obtained from the saphenous vein (Table III). The elapsed time between samplings was between thirty and forty-five minutes, and the doses of insulin and glucose were 0.1 unit per Kg. body weight and 100 gm., respectively. In each experiment, the pre-existing arteriovenous difference was reduced to insignificance following the infusion of glucose and insulin.

Eight normal young men have been investigated with regard to the effects of nutritional state on UFA concentrations in blood drawn from antecubital veins. The subjects received no food after 6:30 P.M. on the day preceding the test period, and blood samples were drawn at about 9:00 A.M. and at hourly intervals thereafter until noon. The nutrients to be tested were fed immediately after the initial blood sample was drawn, each accompanied by approximately 300 ml. of water. On different days, the subjects received glucose, 50 gm., dl-alanine, 50 gm., l-glutamic acid, 50 gm., and l-leucine, 25 gm. On another day, they fasted, receiving only the 300 ml. of water. The amino acids were found to be somewhat unpalatable in pure form, but were in general well tolerated. In three subjects there was mild gastric distress after alanine. The time course of the observed UFA concentrations is given in summary form in Figure 1. The difference between each measured UFA concentration and the baseline value at 9:00 A.M. has been determined for each subject, and comparable values averaged over all subjects. The figure indicates that the feeding of glucose or of any one of the amino acids reverses the normal upward trend of the plasma UFA concentration for a period of roughly two hours. It also appears that glucose is more effective in this respect than are the amino acids. These data have been submitted to statistical analysis, with the finding

TABLE III

Abolition of A-V differences in UFA concentration by intravenous glucose and insulin *

Patient	Venous site	Glucose and insulin	UFA (*mEq./L.*)		
			Arterial	Venous	Diff.
J. B.	H.V.	Before	.68	.63	.05
		After	.10	.10	.00
W. H.	H.V.	Before	.89	.78	.11
		After	.25	.25	.00
A. J.	H.V.	Before	.42	.31	.11
		After	.08	.08	.00
G. E.	S.V.	Before	.46	1.09	−.63
		After	.16	.19	−.03
R. S.	S.V.	Before	.39	.82	−.43
		After	.22	.20	.02
I. S.	S.V.	Before	.36	.50	−.14
		After	.13	.12	.01

* Samples from hepatic (H.V.) and saphenous (S.V.) veins. The figures are means of duplicate analyses.

that the difference between the course of UFA concentrations after feeding any one of the nutriments and that observed during fasting is of significance (p less than 0.01). The greater effectiveness of glucose as compared with the amino acids is of borderline significance (p less than 0.05 for each comparison).[2]

DISCUSSION

With the use of a new analytical procedure which is superior in precision, specificity, and ease of performance, to the method formerly employed, it has been possible to confirm and extend earlier observations on the physiologic behavior of unesterified fatty acids in human blood plasma. In subjects undergoing cardiac catheterization, studies of arteriovenous differences leave no doubt that fatty acids in the form of soluble soaps are entering the circulation from the adipose tissue (this being the only tissue drained by the saphenous vein that could conceivably be the source of the UFA), and are being extracted from the blood by the myocardium and by the viscera whose collective venous drainage is available for sampling in the hepatic vein. The extraction in the viscera is in all probability greater than that indicated by the measured A-V difference, because omental fat may be expected to be adding UFA to the blood within this enteric circulation at the same time that other tissues are extracting it. That skeletal muscle may also extract UFA is almost as certain on the basis of the measured A-V differences in the samples submitted by Drs. Andres and Zierler. Their preparation of human forearm does not permit certain knowledge that no venous drainage from adipose tissue is included in the samples collected, and the addition of UFA to the blood by adipose tissue in a quantity exceeding that being simultaneously extracted by muscles is believed to be the explanation of the small number of negative A-V differences encountered. The attempt to demonstrate A-V differences across the kidney was perhaps foredoomed to failure, as the renal blood flow is so large relative to metabolic demands. From the average renal A-V oxygen difference noted by Cargill and Hickam (4), and using the same assumptions as in previous calculations, it is found that if kidney were to obtain all of its energy by the oxidation of unesterified

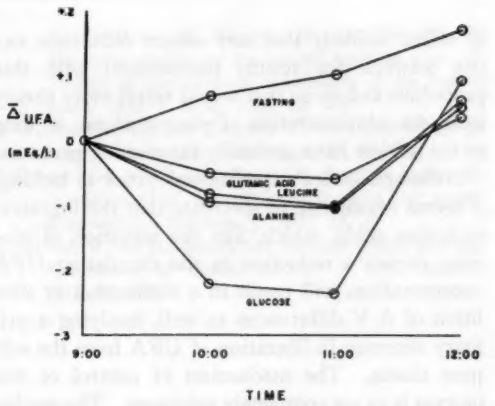

FIG. 1. EFFECT OF FEEDING ON UNESTERIFIED FATTY ACID

The average change in UFA concentration in the antecubital vein, the reference being the basal value at 9:00 A.M., is plotted as a function of time. All feedings were given immediately after the initial blood sample was obtained.

fatty acids, an A-V difference of only 0.05 mEq. per L. would be expected. The values encountered are consonant with the belief that unesterified fatty acid as a substrate is available to renal tissue, but the present data are insufficient to prove that extraction occurs at all, and certainly permit no quantitative conclusions.

A matter of importance is the finding that after administration of glucose and insulin to human subjects, there occurs, coincident with a decreased arterial level of UFA, complete cessation of net transport of UFA as indicated by the abolition of A-V differences. The events observed indicate that the transport of fat as UFA is controlled primarily by the adipose tissue, and that when glucose is made available, there is first a decrease in the liberation of UFA from depot fat, a resultant drop in arterial UFA concentration, and then a cessation of extraction by other tissues. The reverse sequence is not reasonable, as in that case the arterial level would be expected to rise as tissue extraction was halted. A similar direct relationship between arterial level of substrate and the tissue extraction has been observed by Bing, Siegel, Vitale, Balboni, Sparks, Taeschler, Klapper, and Edwards (5) in the case of the non-lipid substrates that they have studied extensively in the human myocardium *in vivo*. In the present studies the combination of glucose and insulin has been employed in order to minimize the time that the subject must spend with the catheter in place.

[2] The author is indebted to Mr. Felix Moore, Biometrics Research Section, National Heart Institute, for guidance in this analysis.

It seems unlikely that any major difference exists between the results encountered with this procedure and those that would result more slowly from the administration of glucose alone, so long as the subject has a normally functioning pancreas.

Although direct experimental proof is lacking, it seems reasonable to speculate that the ingestion of amino acids, which, like the ingestion of glucose, causes a reduction in the circulating UFA concentration, will result in a diminution or abolition of A-V differences as well, implying a primary decrease in liberation of UFA from the adipose tissue. The mechanism of control of this process is as yet completely unknown. The mechanism, whatever its nature, appears to be activated by some receptor system capable of detecting the availability of oxidizable, energy-yielding substrates even though these differ metabolically as much as do glucose, alanine, glutamic acid, and leucine. The last is of special interest, as a representative of the ketogenic amino acids, whose intermediary metabolism is believed to have nothing in common with the metabolism of glucose until degradation has proceeded to the level of acetate. The availability of calories from non-fat sources appears to be so far the chief determinant of plasma UFA concentrations; any operation making calories more available results in a decrease, whereas the opposite causes an increase. The exception appears to be the response to epinephrine, which has been shown (1, 2) to produce an immediate rise in UFA levels. The effect of epinephrine is of particular interest in view of the older work, cited by Wertheimer and Shapiro in their excellent review (6), related to the possibility of nervous control of the function of adipose tissue.

Consideration of the present data leads to two inferences which are being tested in current experimental work. First is the almost inevitable conclusion, derived from the measured A-V differences, that the turnover time of an isotopic label in the plasma UFA fraction should be extremely short. Second is the expectation of elevated plasma UFA concentrations in diabetic patients who are not under insulin control, a clinical situation in which non-fat calories are less than normally available. It is of interest that Dangerfield and Smith (7) some time ago reported on changes in the electrophoretic pattern of plasma taken from acute diabetic acidosis, changes which resemble closely those produced by the addition of sodium oleate to serum *in vitro* (8).

Another reasonable inference to be drawn from these data, but concerning which there are as yet no direct experimental measurements, is that the fatty acids in the unesterified fraction, insofar as they are derived from the adipose tissue, may be expected to have the same chemical characteristics as regards chain length and unsaturation as do the fatty acids of the depot fat. The depots of higher mammals are, in general, made up primarily of the glycerides of stearic, palmitic, and oleic acids. The assumption of an average molecular weight of 275 for the unesterified fatty acids of blood plasma is based on this inference, and if it should be proved incorrect, the values of oxygen extraction ratio given in this and the preceding paper will require appropriate revision.

SUMMARY

Employing a newly developed analytical procedure for the determination of the concentration of unesterified fatty acid (UFA) in blood plasma, it has been possible to demonstrate in fasting human subjects arteriovenous differences indicative of a net transport of fatty acid from adipose tissue to myocardium, skeletal muscle, and viscera. The administration of glucose and insulin causes a sharp decrease in UFA concentrations and an abolition of the arteriovenous differences. Alanine, leucine, and glutamic acid administered by mouth to fasting humans have an effect like that of glucose in reducing UFA concentrations, though the decreases are less dramatic. It is suggested that adipose tissue responds, through some mechanism sensitive to the availability of non-fat calories, by liberating more or less UFA into the blood and so maintaining a "caloric homeostasis."

ACKNOWLEDGMENT

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SODIUM TRANSPORT ACROSS THE SURFACE MEMBRANE OF RED BLOOD CELLS IN HEREDITARY SPHEROCYTOSIS [1]

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The erythrocyte of hereditary spherocytosis (HS), compared with the normal human erythrocyte, is characterized by an increased thickness: diameter ratio, a greater tendency to hemolyze in hypotonic solutions of sodium chloride (osmotic fragility), and a higher rate of hemolysis when incubated at body temperature (spontaneous autohemolysis) (1). Evidence of abnormalities in the glycolytic cycle of the HS cell has recently been presented (2) and corroborated (3). The *in vitro* movement of sodium ion (Na) across the surface membrane of washed erythrocytes from one patient with HS has been quantitated (4): the rate of extrusion was abnormally high both before and after splenectomy. A normally functioning glycolytic cycle has become an experimentally well established requisite for normal cation transport [3] across the surface membrane of erythrocytes (6). Cation transport is required to maintain the low Na and high potassium (K) concentrations in human red cells, as they contrast to plasma Na and K concentrations. Consequently this study was planned to determine:

1) whether HS red cells demonstrate *in vitro* Na transport rates that vary from normal to the same degree for each patient; and
2) how the variation from normal relates to other characteristics of HS cells.

Studies on a total of 14 individuals are presented: Cases 1 through 10 are splenectomized patients, with complete hematologic recovery by routine testing (hemoglobin, hematocrit, and reticulocyte count), whose erythrocytes continue to demonstrate the characteristics of HS. Cases A and B are hematologically normal controls; duplicate Na transport studies were done on their red cells to determine reproducibility. Cases C and D are hematologically normal controls splenectomized in the past for traumatic splenic rupture and idiopathic thrombocytopenic purpura, respectively.

Na transport measurements were done on red cells from all cases presented. Determinations of osmotic fragility and rate of autohemolysis were done on all cases except C and D, and were performed on the same day as the transport study unless otherwise noted in Table I. Correlations were sought among the values for rate of Na transport, osmotic fragility, and rate of autohemolysis.

METHODS

Design of system for in vitro measurement of rate of Na transport. Related experimental schemes have been described by Sheppard and Martin (7), Raker, Taylor, Weller, and Hastings (8), Harris and Maizels (9), Solomon (10), and Love and Burch (11). The method used in this study was a composite, striving for minimal cell manipulation prior to addition of isotope. In each case, 40 ml. of venous blood were drawn through a No. 18 needle into a 50-ml. syringe containing 8 mg. heparin sodium in 0.8 ml. of isotonic sodium chloride solution. The subjects were under no dietary restrictions. Blood was transferred immediately to a siliconed 250-ml. Erlenmeyer flask containing 100 mg. glucose (more than sufficient to maintain an adequate concentration of glucose in the blood in the presence of leukocytes throughout the experiment [12]). The flask was immediately fixed to a shaker platform enclosed in an air incubator with temperature controlled to 37.5 ± 0.5°C. Agitation of the system was horizontal

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[3] "Transport" has been defined as movement against a gradient, implying "the use of an energy supply other than, or additional to, thermal agitation" (5). Since it remains to be shown precisely how energy from glycolysis relates to cation carrier mechanisms, in this paper the more general application of the term "transport" is used: movement against gradients of concentration and electrochemical potential. "Transfer" indicates movement in any direction.

TABLE I

Summary of hematological and biochemical data

Case (Age in years)	Date of splenectomy	Date of experiment	$k_{ab}\frac{1-H}{H}$	Average $[Na]_{plasma}$ *mEq./L.*	Na Transport as: mEq. / L. cells × hrs.	Na Transport as: mEq. × 10^{15} / μ^3 × hrs.	Initial total $[Na]_{cell}$ *mEq./L.*	Final total $[Na]_{cell}$ *mEq./L.*	Rapidly exchangeable $[Na]_{cell}$ *mEq./L.*	Rapidly exchangeable $[Na]_{cell}$ / Average total $[Na]_{cell}$	Thickness / Diameter	50% O.F. % *NaCl*	A.H. at 48 hrs. % *hemolysis*
1 (58)	1953	2/27/56	.044	138.6	6.1	4.1	6.6	6.4	7.1	1+	.32	.67	37.4
2 (33)	1955	5/28/56	.040	142.5	5.7	4.0	5.7	5.2	5.3	.97	.34	.60	14.2
3 (36)	1945	2/9/56	.039	133.3	5.2	3.4	8.8	8.8	7.0	.80	.34	.64 5/23/52	22.4
4 (43)	1950	3/16/56	.038	130.0	4.9	3.2	9.6	8.8	7.7	.84	.33	.63 8/4/54	19.0 2/21/56
5 (7)	1954	5/31/56	.032	140.8	4.5	3.1	6.1	6.9	6.8	1+	.33	.66	23.7
6 (19)	1944	6/9/56	.032	142.7	4.6	3.2	8.3	7.8	6.9	.86	.33	.68 8/21/52	21.3
7 (25)	1941	5/22/56	.029	139.8	4.0	2.6	11.1	10.4	8.2	.76	.30	.61 8/26/54	21.8
8 (58)	1946	5/29/56	.029	140.2	4.1	2.8	7.0	6.9	5.8	.83	.33	.58 4/15/47	6.7
9 (16)	1952	2/14/56	.028	144.5	4.0	2.7	8.3	8.0	6.8	.83	.33	.66 2/6/54	14.3
10 (17)	1940	4/27/56	.026	135.7	3.5	2.4	9.5	9.0	6.9	.74	.33	.66 2/23/54	20.1 4/12/55
A (30)		2/8/56	.027	140.5	3.8	2.3	8.0	8.0	6.4	.80	.28	.53 6/13/56	3.5 6/13/56
A′		4/5/56	.027	141.1	3.8	2.6	7.0	7.0	5.8	.83	.30		3.2 6/14/56
B (30)		3/21/56	.025	137.8	3.4	2.3	8.0	7.4	6.4	.83	.31	.56 6/19/56	4.4 6/20/56
B′		4/20/56	.023	138.5	3.2	2.1	7.4	7.2	5.8	.79	.28		3.7 6/20/56
C (20)	1954	4/17/56	.020	137.5	2.8	1.8	10.9	9.9	7.9	.76	.25		
D (51)	1955	3/29/56	.021	135.3	2.8	1.8	10.1	9.2	7.1	.74	.23		

at 150 strokes per minute over a 2-cm. traverse. A constant atmosphere of 5 per cent CO_2 and 95 per cent O_2 was maintained in the flask. When constant pH[4] was reached, approximately 0.3 microcurie of Na^{22} [5] contained in 0.4 ml. of isotonic sodium chloride solution was added to the blood, the flask was returned to the shaker, and the experiment timed from the moment of addition of isotope.

Sampling. About 4.5 ml. of whole blood for determinations of cell and plasma radioactivity and total Na (Na^{22}+Na^{23}) concentrations were pipetted from the flask at 12 minutes and at hourly intervals thereafter until the concentration of radioactivity in the cell fraction reached a plateau (Figure 1). Duration of experiments ranged from 6 to 7 hours, with plateau generally not being reached until after 5 hours. Duplicate microhematocrit (13) determinations were done on each sample, and then duplicate aliquots of whole blood were immediately pipetted into 10 by 60 mm. glass tubes with a pipette recalibrated to deliver 2.00 ml. of blood. The aliquots were centrifuged at 1,710 R. C. F. for 30 min., following which the supernatant plasmas were pipetted off and combined for subsequent measurements of total Na and of radioactivity. The surface of each packed cell mass, plus the tube interior above the surface, were carefully washed free of plasma with four changes of isosmotic (approximately 5.2 per cent) glucose solution, using a Pasteur pipette and exercising great care not to disturb the red cell mass. The subsequent determination of radioactivity remaining in the packed cell mass was not significantly altered when three to six such changes of wash solution were employed. Four changes were arbitrarily chosen.

Radioactivity determinations. Duplicate 0.5-ml. aliquots of plasma and the duplicate aliquots of packed cells were counted at constant volume in a well-type scintillation counter to within 1 per cent probable error. In more than 100 sets of duplicate packed cell counts, all but 3 duplicates agreed within 2 per cent, and those 3 agreed within 5 per cent.

Corrections for trapped plasma. Using I^{131} human serum albumin as a plasma tracer, we confirmed the finding (14) that the volume of plasma trapped among HS cells spun at 10,000 RPM in a capillary tube (13) is about 2.5 times that trapped with normal cells. The true value of hematocrit,[6] within the error of the method and within the hematocrit range of this study (from 36 to 46 per cent), may be obtained by subtracting 1 from the capillary hematocrit reading for normal cells, and subtracting 2.5 from the reading for HS cells. In similar fashion the corrections for plasma trapped in the packed cell mass in 10 by 60 mm. tubes under the conditions of the experiment were obtained: 0.032 ml. plasma per ml. normal cells, and 0.038 ml. plasma per ml. HS cells. These corrections were applied when calculating counts per minute (CPM) per ml. cells, and when determining total Na concentrations of cells and mean corpuscular volume (MCV).

Total Na (Na^{22}+Na^{23}) determinations. A flame photometer (lithium internal standard) of the type described by Berry, Chappell, and Barnes (15) was used. Aliquots of plasma were diluted 1:500, and the packed cell masses after counting were diluted to 100 ml. The feasibility of employing red cell hemolysate for flame photometry is recognized (16–18). Small downward corrections of cell Na values in the presence of K were required. The agreement between determinations of cell Na concentration done on the duplicate aliquots was, to 2 standard deviations (2 S.D.), within 0.3 mEq. per L. cells. Variability of plasma Na concentration over the course of each experiment was never over 2.5 per cent, and was generally much less.

Hemolysis during Na transport experiments. Determined as oxyhemoglobin, this was slightly over 0.5 per cent of the cell mass in four instances. The per cent hemolysis bore no constant relation to cell Na transport rate.

Na transport rate calculations for red cells. Solomon's notation (10) is employed. Let the movement of isotope be described:

$$\frac{dq}{dt} = k_{ab}\, p \frac{v_p}{v_q} - k_{ba}\, q \frac{v_p}{v_q} \qquad (1)$$

where $q = [Na^{22}]_{cells}$ and $p = [Na^{22}]_{plasma}$

t = time

v_q = cell mass volume = H = hematocrit

v_p = plasma volume = 1 − H.

Letting $q_\infty = [Na^{22}]_{cells}$ at plateau, the plot of $\ln\left(1 - \frac{q}{q_\infty}\right)$ against time (Figure 2), thus minimizing errors involved in single determinations of q, should yield a straight line if the assumptions leading to construction of Equation 1 are correct. If slope equals T, then:

$$k_{ab} \frac{1 - H}{H} = \frac{-Tq_\infty}{p_0} \qquad (2)$$

where $p_0 = [Na^{22}]_{plasma}$ at time zero.

$k_{ab} \frac{1 - H}{H}$ represents the fraction of the total Na concentration of plasma (Na^{22}+Na^{23}) which transfers into one liter of cells in 1 hour. If a steady state obtains, this is also the quantity of Na transported out of 1 liter of cells per hour.

p drops less than 4 per cent during the course of an experiment, and since p_0 is a critical value in computing k_{ab}, we have employed the average value of p during each experiment to represent p_0. We have also used average p instead of p_∞ in the calculation of "rapidly exchangeable"

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[4] Beckman model G glass electrode pH meter, open cup. Readings by this method are slightly higher than those obtained in a closed system. True pH of 7.6 was not exceeded (9). pH dropped about 0.2 unit during each experiment.

[5] Identity and purity of the radioactive isotope were confirmed through the courtesy of H. Mermagen and D. H. Maillie, Atomic Energy Project, University of Rochester.

[6] The term "hematocrit" is used by some authors to indicate the calibrated tube in which blood is centrifuged in order to determine the fractional volume of blood occupied by red cells. "Hematocrit" in general clinical usage means this fraction expressed as per cent, and this meaning is intended in this paper.

cell Na (10):

$$[Na]_{exch.} = \frac{q_\infty}{p_\infty/[Na]_{plasma}} \quad (3)$$

where $p_\infty = [Na^{22}]_{plasma}$ at plateau.

It must be pointed out that cell Na concentration dropped slightly over the course of most of the experiments (see Table I). This is a recognized phenomenon (9–11) and detracts from the accuracy of the transport calculations, but does not invalidate them.

Expression of transport rate in terms of surface area of cells rather than volume of cells. Movement of Na across the red cell membrane in terms of cell surface area may permit a more valid comparison of one cell type with another when area:volume ratios differ (9). Convenient rather than highly accurate devices were employed in this conversion. We used Emmons' formula (19) for cell surface area, which assumes the cell is a truncated cylinder:

$$\text{Area} = 2\pi r(r + h) \quad (4)$$

where r = cell transverse diameter/2

h = cell thickness = $MCV/\pi r^2$.

It is assumed that the error introduced by using this formula to calculate surface area is no greater when applied to slightly spheroidal (*i.e.*, biconvex) HS red cells than when applied to biconcave normal red cells.

The following expression then may be derived:

Na transport as mEq./μ^2 of surface area × hrs.

$$= \frac{\left(k_{ab}\frac{1-H}{H}\right)([Na]_{plasma})(MCV)}{(10^{15})(\text{Area})}. \quad (5)$$

Erythrocyte diameter was measured by the Haden-Hausser erythrocytometer[7] with a reproducibility of ±0.1μ. The hematocrit figure employed to obtain MCV was the average true hematocrit of the experiment, and remained constant to within ±3 per cent (2 S.D.). Red cell counts as performed yielded the greatest source of possible error, being accurate only to within ±9 per cent (2 S.D.) as computed by the formula of Berkson, Magath, and Hurn (20). Obviously these errors could yield considerable variation in the rates calculated using Equation 5, but in practice the reproducibility was good (see Table I).

Determination of osmotic fragility (O.F.) and rate of autohemolysis (A.H.). The procedures used are described by Young and his associates (21, 22). The "50% O.F." figure in Table I is the concentration of sodium chloride solution in which 50 per cent of a cell population lyses after the cells have incubated for 24 hrs. at 37°C. in autogenous serum. The figure for "A.H. at 48 hrs." is per cent hemolysis occurring in a cell population during 48 hrs. incubation at 37°C. in autogenous serum.

RESULTS

The pertinent data are summarized in Table I. Na transport rates for normal erythrocytes (2.8 to 3.8 mEq. per L. cells × hrs.) (cases A, A′, B, B′, C, and D) fit exactly with previously reported ranges (10, 11). Transport rates for the HS erythrocytes (cases 1 through 10) vary from high normal to considerably elevated figures. No advantage is gained in expressing Na transport in terms of surface area rather than volume: Both methods demonstrate equally well the difference between HS and normal red cells. Case histories, response to splenectomy, and the values reported here for cell thickness:diameter ratio, autohemolysis, and osmotic fragility leave no doubt that cases 1 through 10 correspond to the syndrome of hereditary spherocytosis; although patients 7, 9, and 10 have no similarly affected relatives, including both parents of each, among those tested.

Inspection of the data reveals no significant correlation between Na transport rates and total Na concentrations in cells, nor between Na transport rates and ratios of "rapidly exchangeable" Na:total Na concentrations in cells. The following scattergrams were constructed using the data from cases 1 through 10, but are not shown here:

Na transport rate versus plasma Na concentration;
Na transport rate versus osmotic fragility;
Na transport rate versus autohemolysis rate;
Total Na concentration in cells versus osmotic fragility;
Total Na concentration in cells versus autohemolysis rate;
Osmotic fragility versus autohemolysis rate;
Ratio of "rapidly exchangeable" Na:total Na concentration in cells versus osmotic fragility;
Ratio of "rapidly exchangeable" Na:total Na concentration in cells versus autohemolysis rate.

None of these scattergrams revealed significant correlation; but it must be noted that the number of cases studied was small. In this laboratory (22), determinations on a much larger number of HS patients have demonstrated a positive correlation between osmotic fragility and rate of autohemolysis. HS erythrocytes cannot here be distinguished statistically from normal erythrocytes either in terms of total Na concentration or in terms of the "rapidly exchangeable" Na:total Na concentration ratio. Routine hematologic data (hemoglobin, hematocrit, and reticulocyte count)

[7] Distributed by Clay-Adams, New York, New York.

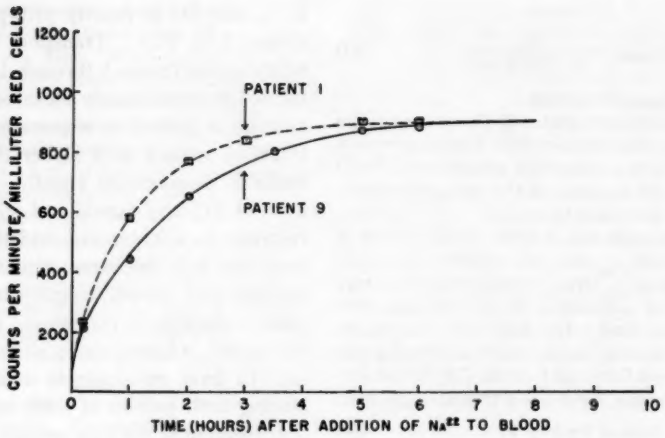

FIG. 1. IN VITRO RISE OF RADIOACTIVITY IN RED CELLS FROM TWO HS PATIENTS

These two patients represent opposite extremes of the scale of Na transport rate, patient 9 being at the upper limit of normal. The superimposition of plateaus is coincidental.

obtained before splenectomy were available on cases 4 through 10; no correlation exists between the apparent severity of the disease prior to splenectomy and the data reported here.

The average total initial Na concentration in cells, for the 10 HS patients, is 8.1 mEq. per L. cells; for the 6 determinations on the controls (cases A through D) the average concentration is 8.6. The average "rapidly exchangeable" Na concentration in cells for the 10 HS patients is 6.8; for the 6 controls the figure is 6.6. Combining these two sets of non-dissimilar averages one obtains an overall (16 determinations) average "slowly exchangeable" Na fraction of 19.3 per cent. Love and Burch's comparable figure (as calculated in [23]) is 17.8 per cent, and Gold and Solomon's figure (23) is 20.1 per cent.

DISCUSSION

There is satisfactory evidence that the defects of HS cells basically responsible for their shortened life span in the untreated (non-splenectomized) patient arise intrinsically (24). There is no evidence directly relating the known abnormalities of the HS cell to its premature *in vivo* destruction. The characteristically delayed passage of these spheroidal cells through the spleen of either a normal or an HS patient results in an accentuation of the cells' susceptibility to hypotonic hemolysis *in vitro* (25), but such low levels of tonicity are not realized *in vivo*. Selwyn and Dacie (26) have demonstrated that the hemolysis produced by the incubation of sterile HS blood is not attendant upon progressive erythrocyte swelling, and Crosby (27) cites evidence against the concept of hypotonic hemolysis being the result of "bursting" of the red cell from intracellular pressure.

The premature demise of the HS cell may be related to abnormal glycolysis (2, 3, 28). There exists sufficient evidence (2, 3) to conclude that the incorporation of extracellular phosphate into ATP (adenosine triphosphate) in the HS red cell deviates significantly from normal. The present study indicates that the rate of transfer of Na across the HS cell surface membrane is higher by a variable amount than the rate across the membrane of the normal red cell; however, the total Na concentration (26, and this paper) of the HS cell is essentially normal. Transmembrane concentration gradients of both Na and K are reduced in the cold-stored normal erythrocyte (29), in HS or normal red cells incubated over 24 hrs. at 37°C. (26), in sickled cells (30, 31), in red cells exposed to inhibitors of glycolysis (32), and in mammalian red cells exposed to a variety of nox-

ious stimuli (33). Disordered phosphate turnover rates are not peculiar to the HS cell alone (2, 34), and it may well be that pathologically altered glycolytic pathways and abnormal cation transport rates (4) characterize erythrocytes destined for early destruction by a variety of forces (28). It is reasonable to imagine that specific enzyme defects or deficiencies in specific disease states might lead to this "final common pathway." A more general example of this is the aging normal erythrocyte, whose total enzyme activity level, it has been suggested (35), decreases exponentially with time. Pyruvic phosphoferase, required in the phosphorylation of ADP (adenosine diphosphate) to ATP, is activated by potassium ion, but the typically low K concentration of HS red cells probably does not contribute to their abnormal phosphate turnover (36).

The exact circumstances surrounding the early death of the HS cell in non-splenectomized patients are still not known. Selwyn and Dacie's work (26) showed that changes in Na concentration in HS cells during *in vitro* incubation at 37°C. did not vary significantly from those occurring in normal human erythrocytes, even though incubation was carried out for 48 hours without added glucose. They suggested that "degenerative changes in the cell membranes" accounted for the increased rate of hemolysis of incubated HS cells, and linked this finding to prior speculation (37) on the role of the spleen in HS. The abnormal shape of the HS red cell certainly suggests a membrane defect, but further evidence exists: Prankerd, Altman, and Young found that the incorporation of radiophosphate into ATP and 2,3-diphosphoglycerate of the stroma of HS cells was lower than normal (2). Thus, a red cell membrane abnormality undoubtedly exists, but one must keep in mind that simplified mathematical analyses of isotope movement across cell surfaces, as in the present paper, are primitive approximations to probably complicated distributions involving total cell metabolism.

At present one cannot state with certainty how an accelerated movement of Na across the HS cell membrane is related to the aberrant behavior of these red cells *in vivo* and *in vitro*. The mechanism by which cations are transported across cell membranes is not known. Maizels has stated (6) that majority opinion favors "the view that Na and

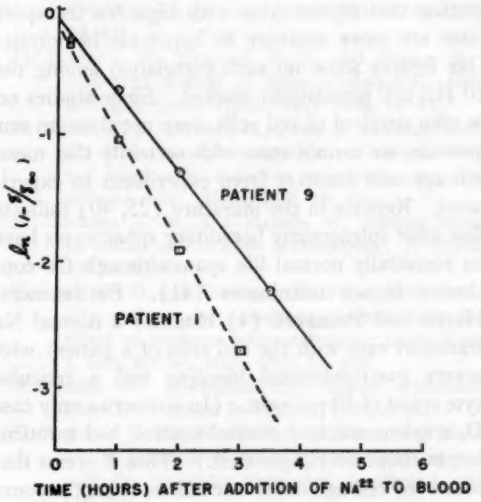

FIG. 2. PLOT OF THE LOGARITHMIC FUNCTION OF q (COUNTS PER MINUTE PER ML. OF RED CELLS) AGAINST TIME: THE TWO PATIENTS ARE THOSE OF FIGURE 1

The slopes (T) of these plots employed in Equation 3 are actually calculated by the method of least squares from the original data. The plots are visual checks on the validity of the slope calculation. q_∞ represents counts per minute per ml. of red cells at plateau.

K are transported in complex combination with a carrier, presumably lipoid or lipoid soluble." There is evidence (38) that active fluxes of red cell Na and K are linked in some manner. Also, recent work by Glynn (39) indicates that, under certain circumstances, the movement of Na *in vitro* out of washed erythrocytes from normal humans may have a passive component not dependent on glycolysis. Should this passive Na flux exist also under the more physiologic experimental conditions of this paper, an accelerated passive efflux from HS cells could explain our findings. This explanation would also be consistent with the demonstration (26) that the rate of disappearance *in vitro* of glucose from serum containing HS cells is normal.

We chose splenectomized HS patients for the study presented in order that mean cell age might vary as little as possible from patient to patient. Harris and Prankerd (4) suggested that the younger cells in an erythrocyte population display a more active Na transport across their membranes. They also interpreted their data as sug-

gesting that erythrocytes with high Na transport rates are more sensitive to hypotonic hemolysis: Our figures show no such correlation among the 10 HS cell populations studied. Since studies on *in vivo* survival of red cells were not done on our patients, we cannot state with certainty that mean cell age was constant from experiment to experiment. Reports in the literature (25, 40) indicate that after splenectomy hereditary spherocytes have an essentially normal life span, although the conclusion is not unanimous (41). Furthermore, Harris and Prankerd (4) obtained a normal Na transport rate with the red cells of a patient with severe gastrointestinal bleeding and a reticulocyte count of 10 per cent. (In our series only case D, a splenectomized normal control, had a reticulocyte count over 2 per cent.) Thus it seems that mean red cell age differences alone cannot account for the range of Na transport values in Table I.

Young has commented previously (42) on the absence of hematologic abnormality in both parents and all tested relatives of our cases 7, 9, and 10 (his patients WE, EH, and VO). Splenomegaly, anemia, reticulocytosis, and hyperbilirubinemia were absent, and determinations of osmotic fragility were normal. This suggested either a minor degree of penetrance in a carrier parent (assuming HS is inherited as a Mendelian dominant), or the possibility that the patients represented gene mutations. Our Table I indicates that the cells of cases 7, 9, and 10 have the lowest transport constants of the HS group. Thus is strengthened the suspicion (2, 42) that the clinical syndrome of hereditary spherocytosis is a composite of genetically determined biochemical abnormalities in red cells, not all of which abnormalities need be present in one patient.

The values for total Na concentration in normal red cells are lower than some recently published (16, 23). The reason for this is not clear, since we too analyzed complete cell aliquots, not discarding the top Na-rich layer of cells. However, our figures serve chiefly to verify steady state conditions, and to provide comparative Na concentrations. Gold and Solomon (23) discovered that the red cells of two untreated (non-splenectomized) HS patients demonstrated a higher proportion than normal of "rapidly exchangeable" Na, thereby suggesting "that the fraction of slowly exchanging Na may increase with the age of the red cell." The significance of slowly and rapidly exchanging Na fractions in red cells remains to be determined. Their existence is demonstrated experimentally by the ratio of Na specific activity in red cells to that in plasma, this ratio reaching a sub-unity value at plateau and remaining there at least to the 30-hr. point (23). Our work confirms this existence, but reveals no statistical difference ($P > 0.1$) between normal red cells and post-splenectomy HS cells in regard to the magnitude of the fractions.

SUMMARY

1. *In vitro* Na transport rates were determined for the red cells of 10 splenectomized patients with hereditary spherocytosis (HS), and 4 normal controls. Osmotic fragility and rates of spontaneous autohemolysis were also determined on the red cells of these 14 individuals.

2. Na transport rates of HS cells ranged from high normal to considerably elevated values. The abnormal rates are consistent with the fact that glycolytic pathways deviate from normal in HS red cells; and erythrocyte cation transport is dependent on glycolysis. The possibility also exists that increased passive Na efflux, not linked to glycolysis, may be responsible for the abnormal rates.

3. Na transport rates of red cells of 3 HS patients, each of whom had hematologically normal parents, were the lowest of the 10 HS cases studied. This finding strengthens the suspicion that HS is a composite of genetically determined biochemical abnormalities.

4. The existence of slowly and rapidly exchanging Na fractions in red cells was confirmed. Normal red cells and post-splenectomy HS cells did not differ in regard to the magnitude of these fractions.

5. No significant correlations were found among Na transport rates, osmotic fragility, autohemolysis rates, total Na concentrations, and "rapidly exchangeable" Na concentrations of HS cells. Total Na concentrations and concentrations of "rapidly exchangeable" Na in HS cells were not distinguishable from the corresponding concentrations in normal erythrocytes.

6. Although experimental evidence indicates that the erythrocytes of HS patients have abnormally functioning surface membranes and altered intracellular glycolytic pathways, the precise mech-

anisms leading to the shortened survival of these cells in non-splenectomized patients are not known.

ACKNOWLEDGMENT

Determinations of osmotic fragility, rate of autohemolysis, and red cell transverse diameter (by erythrocytometer) were performed by Miss Mary Jane Izzo. The author is grateful to Dr. Lawrence E. Young for access to this group of patients previously studied in this laboratory, and for his advice in the preparation of the manuscript.

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THE ACUTE CALCIFICATION OF TRAUMATIZED MUSCLE, WITH PARTICULAR REFERENCE TO ACUTE POST-TRAUMATIC RENAL INSUFFICIENCY [1]

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Previous studies of acute post-traumatic renal insufficiency in man have shown that plasma concentrations of inorganic phosphate and calcium were inversely related (2), and that those patients with extensive devitalization of muscle had the most acute and extreme hyperphosphatemia and hypocalcemia (3). When calcium was injected intravenously into such patients, the elevation of plasma concentration was very transient, and the rate at which the injected calcium escaped from the plasma appeared to be proportional to the degree of the pre-injection hypocalcemia and hyperphosphatemia, which, in turn, was roughly proportional to the degree of muscle damage. In order to maintain plasma calcium at a desired level, it was necessary to infuse calcium continuously. The purpose of the maintenance of a near-normal calcium level was to antagonize the toxic effects of hyperpotassemia, and in this respect prolonged infusions were quite effective; however, the desirability of the procedure was questioned because the fate of the infused calcium was unknown, and the quantities employed might be harmful if deposited in certain viscera.

At first consideration it might be assumed that the calcium which escaped from the plasma was deposited in bones. However, this would not explain the difference in the rate of disappearance of calcium from the plasma of patients with and without muscle injury, since the bones were similar in both. An alternate possibility is that some of the calcium was deposited in the damaged tissue, a phenomenon well recognized in chronic states but not known to occur acutely. It is to test this possibility that the present experiments are designed.

[1] Previously published in abstract form (1).

EXPERIMENTAL PROCEDURES AND METHODS

Dogs 1 and 2 were given tracer doses of $Ca^{45}Cl_2$ intravenously on day zero, and plasma samples collected daily thereafter were analyzed for radioactivity, sodium, potassium, non-protein nitrogen, total protein, calcium, phosphorus, and pH. On day 3, with the animals under Nembutal® anesthesia, a biopsy specimen was taken from the right forelimb, bilateral nephrectomy was performed, and the large muscles of the right hind limb were severely traumatized with a hammer, care being taken not to strike the femur. On day 6, biopsy specimens of approximately 20 grams were taken from the traumatized muscle of the right hind limb and the untraumatized muscle of a comparable area of the left hind limb.

Dogs 3, 4, and 5 were nephrectomized and traumatized in a similar way, and biopsies were taken on the third day. The muscle specimens were analyzed for calcium and fat only.

The procedure for dogs 6 and 7 was the same as for dogs 1 and 2 except that nephrectomy was not performed. The procedure for dog 8 was the same as for 6 and 7 except that the initial, normal biopsy was omitted and the biopsies of the hind limbs were performed on day 4, 24 hours following injury.

Dogs 9 to 13 were not nephrectomized, the initial biopsies were omitted, and radioactive calcium was not given. Dog 9 received intravenously 1050 ml. of an aqueous solution containing 2.95 Gm. NaH_2PO_4 and 11.7 Gm. Na_2HPO_4 per liter (3.2 mgm. phosphorus per ml., pH 7.4) during the six hours following the trauma and preceding the biopsies. Dog 10 received 1200 ml. of this solution during the six hours following the trauma and an additional 1150 ml. over a six-hour period prior to biopsies the following day. Dog 11 received 600 ml. 0.4 molar $NaHCO_3$ in water during the six hours following the trauma and preceding the biopsies. Dog 12 received 600 ml. 0.1 molar HCl in five per cent glucose during the six hours following the trauma and preceding the biopsies. Dog 13 received 300 μ parathyroid extract (from a lot of known potency) intramuscularly immediately following trauma, and three doses of 200 μ each at two-hour intervals prior to biopsies six hours following trauma.

The tissues from the initial, normal biopsies were weighed, frozen, and saved to be processed with the specimens of traumatized and control muscle obtained on day 6. All specimens were minced and placed in a dry-

TABLE I

Chemical values and radioactivity in plasma and muscle from dogs 1 and 2 *

		Plasma								Muscle			Fat-free wet muscle							
	Day	CPM ×10⁴ per mgm. Ca	Na mEq./L.	K mEq./L.	NPN mgm. %	TP Gm. %	Ca mgm. %	P mgm. %	pH	Muscle sample	H_2O %	Fat %	Na mEq./Gm.	K mEq./Gm.	N mgm./Gm.	P mgm./Gm.	Ca mgm./Gm.	CPM ×10⁴ per mgm. Ca	CPM ×10⁴ per Gm. muscle	Na/K
Dog No. 1	1	22.2	148.5	4.0	37.0	7.0	10.65	6.4	7.40											
	2	12.2	144.4	3.9	34.0	6.9	10.55	7.7	7.36											
	3	7.6	147.7	3.9	36.3	6.6	12.79	7.3	7.33	Normal	76	5.1	.032	.080	30.5	2.25	.090	4.9	0.44	.4
	4	4.8	137.1	4.0	92.0	6.3	12.29	11.2	7.32											
	5	3.3	153.5	6.1	167.8	6.1	12.29	14.3	7.19											
	6	2.7	146.4	6.5	252.0	7.0	12.05	17.5	7.19	Control	80	5.7	.022	.055	22.6	1.50	.080	2.5	0.20	.4
										Trauma	81	7.5	.052	.024	18.6	1.35	.920	5.7	5.23	2.2
Dog No. 2	1	14.6	141.7	4.5	32.4	6.6	11.45	5.7	7.34											
	2	8.9	146.4	3.9	23.3	6.4	12.26	6.3	7.34											
	3	5.4	149.3	4.5	24.2	6.6	13.10	6.0	7.37	Normal	77	6.7	.028	.072	25.8	1.63	.087	2.8	.24	.4
	4	3.8	146.2	5.3	74.0	6.2	12.02	9.1	7.38											
	5	3.3	139.2	5.9	143.0	5.8	12.54	10.7	7.28											
	6	2.4	146.0	7.9	195.0	6.6	13.29	13.0	7.28	Control	74	4.9	.029	.101	35.0	2.06	.089	1.5	.13	.3
										Trauma	77	5.3	.056	.066	31.4	2.12	1.310	3.2	4.20	.8

* Tracer doses of $Ca^{45}Cl_2$ were given intravenously on day zero. On day 3, a muscle sample was obtained by biopsy of the right forelimb, bilateral nephrectomy was performed, and the large muscles of the right hind limb were severely traumatized. Muscle samples were obtained from the area of trauma and a comparable area of the left hind limb on day 6.

ing oven at 87° C. for 72 hours. Preliminary studies revealed that the loss of weight from minced tissues was minimal after 72 hours. Duplicate samples from each dried specimen were digested with nitric acid, heat, and perchloric acid. The digests were suspended in a carrier of normal plasma, and radioactivity was determined by use of a Q gas flow counter for ten-minute periods. All results in tissue digests and plasma were corrected for self-absorption by comparison with a curve constructed using varying amounts of Ca^{45} in normal plasma. The results were expressed as counts per minute per gram of wet, fat-free muscle and as counts per minute per milligram of calcium in plasma and wet, fat-free muscle. Fat was not removed from any muscle because of the likelihood that fluid would be lost from the edematous traumatized muscle, and the fat-free values reported were derived by calculation after the fat content was known from chemical analysis. The tissue concentrations were expressed per gram of wet weight in order that they might be related to wet weight of plasma. All chemical analyses were performed by methods previously reported from this laboratory (4) except fat, which was determined by a modification of the method of van de Kamer, Ten Bokkel Huinink, and Weyers (5). Nitrogen and fat content were determined from a separate sample of dried tissue; the other chemical determinations were performed on the acid digest.

RESULTS

The results for dogs 1 and 2 are shown in Table I. Chemical changes characteristic of the uremic state in dogs were apparent in each plasma sample following nephrectomy. Plasma specific activity (corrected counts per minute per milligram of total calcium) diminished daily as the injected Ca^{45} exchanged with the native calcium in the body pool. The normal muscle, obtained by biopsy of the forelimb prior to other procedures on day 3, yielded chemical values which compare well with those cited by Sunderman and Boerner (6). The control muscle, which was undamaged but was removed on day 6 when the animals were uremic, yielded chemical values which deviated somewhat from normal but showed no large or consistent changes attributable to uremia. The specific activity of the control muscle was lower than that of the normal muscle removed three days previously because the plasma calcium with which it was exchanging had lower specific activity on day 6. The traumatized muscles contained some 26 and 32 times more radioactivity per gram than the respective control muscles. The content of calcium, as measured chemically, was some 12 and 15 times greater in the traumatized than in the control muscles. The specific activity of the calcium was much greater in the traumatized muscle than in the plasma on day 6, indicating that the muscle calcium was derived from plasma immediately following trauma, when the specific activity of plasma was higher, and suggesting that little exchange occurred between the calcium in the traumatized muscle and in plasma.

TABLE II

Effect of trauma on water, fat, and calcium content of muscle *

	Day	Muscle sample	H_2O %	Fat %	Fat-free wet muscle Ca mgm./Gm.
Dog No. 3	3	Control	70	10.5	.090
		Trauma	73	9.5	.798
Dog No. 4	3	Control	66	12.6	.070
		Trauma	69	12.4	.438
Dog No. 5	3	Control	71	8.4	.057
		Trauma	78	6.4	.654

* Dogs 3, 4, and 5 were bilaterally nephrectomized and the large muscles of the right hind limbs were crushed on day zero. On day 3 muscle samples were obtained by biopsy from the area of trauma and a comparable area of the left hind limb.

In dogs 3, 4, and 5 (Table II) the traumatized muscles removed 3 days following nephrectomy contained 9, 6, and 11 times as much calcium as the respective control muscles.

The results for dogs 6 and 7 are shown in Table III. These dogs were not nephrectomized but the other procedures were the same as for dogs 1 and 2. The degree of trauma administered was not regulated in any of the animals, and the change in Na/K ratio is shown as one indication that the trauma was adequate to produce severe changes. In dogs 6 and 7 the plasma chemical changes, the daily diminution in plasma specific activity, and the increased Na/K ratio in the traumatized muscle were similar to the changes in dogs 1 and 2. However, when compared with the control, the traumatized muscle contained only four times the radioactivity and twice the calcium. The specific activity was comparable with that of the plasma on day 4 or 5, whereas the specific activity in the control muscle was comparable with that in plasma on day 6. The determinations of radioactivity in the muscle samples from dog 7 were technically unsatisfactory and are not shown. In dog 8 the traumatized muscle removed 24 hours following injury also revealed four times the radioactivity and twice the chemical calcium of the control (Table III). Again, the specific activity in the traumatized muscle compared best with that in plasma at the time of injury, whereas the spe-

TABLE III

Chemical values and radioactivity in plasma and muscle from dogs 6, 7, and 8 *

		Plasma								Muscle										
													Fat-free wet muscle							
	Day	CPM ×10⁴ per mgm. Ca	Na mEq./L.	K mEq./L.	NPN mgm. %	TP Gm. %	Ca mgm. %	P mgm. %	pH	Muscle sample	H_2O %	Fat %	Na mEq./Gm.	K mEq./Gm.	N mgm./Gm.	P mgm./Gm.	Ca mgm./Gm.	CPM ×10⁴ per mgm. Ca	CPM ×10⁴ per Gm. muscle	Na/K
Dog No. 6	1	14.0	146.0	3.4	35.3	7.7	11.49	4.3	7.40											
	2	7.9	141.0	4.3	30.6	7.8	11.99	5.2	7.38											
	3	7.1	142.9	4.1	30.1	7.5	11.68	4.0	7.36	Normal	74	1.4	.033	.089	32.8	1.70	.072	4.6	.33	.4
	4	6.8	137.7	4.0	24.5	7.5	10.96	3.9												
	5	3.9	137.7	4.5	38.1	7.7	11.37	3.5	7.38											
	6	3.3	131.3	4.5	28.3	7.7	10.70	3.7	7.38	Control	75	0.6	.033	.085	32.4	2.02	.063	2.4	.15	.4
										Trauma	77	1.0	.081	.043	26.0	1.09	.129	4.5	.58	2.0
Dog No. 7	1	20.8	144.6	3.9	30.0	7.9	12.57	3.8	7.42											
	2	6.7	149.8	4.1	29.1	7.4	12.13	4.0	7.44											
	3	3.1	148.9	4.0	27.4	7.6	10.85	4.8	7.40	Normal	75	7.2	.029	.071	28.6	1.77	.053	Unsatisfactory		.4
	4	2.6	143.3	3.6	24.8	7.1	10.70	4.9	7.40											
	5	1.4	136.9	3.8	21.1	6.9	10.55	4.5	7.40											
	6	0.5	140.0	4.2	24.4	7.2	9.24	5.8	7.38	Control	60	18.1	.033	.087	36.3	2.15	.075	Unsatisfactory		.4
										Trauma	74	10.5	.084	.043	24.6	1.26	.141	Unsatisfactory		2.0
Dog No. 8	1	31.6	154.1	3.5	22.5	5.6	10.03													
	2	10.8	153.4	3.5	14.9	6.0	10.70	2.5												
	3	6.5	154.4	3.5	18.3	5.9	10.29	3.7												
	4	4.9	153.3	3.7	14.7	5.9	10.07	4.0		Control	58	18.9	.028	.082	32.7	1.91	.076	3.7	.28	.3
										Trauma	59	19.4	.070	.064	27.8	1.63	.139	6.8	.94	1.1

* Tracer doses of $Ca^{45}Cl_2$ were given on day zero. On day 3, a muscle sample was obtained from dogs 6 and 7 by biopsy of the right forelimb, and the large muscles of the right hind limb of all three dogs were severely traumatized. Nephrectomy was not performed. Muscle samples were obtained from the area of trauma and a comparable area of the left hind limb on day 6, from dogs 6 and 7, and on day 4, from dog 8.

TABLE IV

Chemical values in plasma and muscles from dogs 9 and 10 *

	Plasma			Muscle							
							Fat-free wet muscle				
	Day Hour	Ca *mgm. %*	P *mgm. %*	Muscle sample	H_2O %	Fat %	Na *mEq./Gm.*	K *mEq./Gm.*	P *mgm./Gm.*	Ca *mgm./Gm.*	Na/K
Dog No. 9	1	11.15	5.4								
	2	11.48	4.7								
	3 0900	8.81	19.7								
	3 1100	7.97	15.5								
	3 1300	7.81	11.5								
	3 1500	7.97	9.9	Control	61	13.6	.062	.102	2.25	.078	.6
				Trauma	76	6.3	.099	.033	0.95	.128	3.0
Dog No. 10	1	10.87	4.9								
	2	11.48	5.6								
	3 0900	9.66	18.5								
	3 1100	8.45	20.4								
	3 1500	7.73	15.7								
	4 0800	10.26	2.7								
	4 1100	9.42	11.4								
	4 1500	8.45	10.3	Control	73	5.9	.033	.086	2.01	.062	.4
				Trauma	73	8.0	.079	.046	1.24	.106	1.8

* Dog 9 received a phosphate infusion over a six-hour period following trauma and preceding the biopsies. Dog 10 received a phosphate infusion over a six-hour period following trauma and an additional amount over the six-hour period preceding biopsies the following day.

cific activity in the control muscle was lower than that in plasma 24 hours following injury.

The twofold increase in calcium content of traumatized muscle was consistent in all the nonnephrectomized dogs. The infusion of phosphate for a 6-hour period in dog 9 and for two 6-hour periods in dog 10 (Table IV), the infusion of alkali in dog 11 and acid in dog 12, and the administration of parathyroid hormone to dog 13 (Table V) did not alter the twofold increase in calcium deposition.

All specimens of traumatized muscle appeared edematous, and in eleven of the thirteen the water content, as estimated from the difference between wet and dry weight, was greater than that of the control specimens, but the magnitude of the increase was insufficient for contained plasma to produce the observed alterations in chemical concentration. The calcium concentration in traumatized muscle was greater than that in plasma, and any increase in the plasma contained in muscle could only serve to minimize the increase in muscle calcium. In order for the increase in sodium and the decrease in potassium content to have been the result of an influx of plasma, it would have been necessary for each gram of wet tissue to show an increase of water content averaging 33 per cent (range 0.19 to 0.44, which in most instances would have replaced all the tissue solids), whereas the observed increase of water content averaged 4.7 per cent (range 0 to 0.14). The estimate of the water content of some specimens was unexpectedly low, but these were the specimens containing the greatest amount of fat, and the lower water content of fat may have accounted for the difference. Also, it is possible that the fat interfered with the loss of water during the drying period. These changes were found in both control and traumatized specimens, and the direction of the changes in chemical concentra-

TABLE V

Chemical values in plasma and muscle in dogs 11, 12, and 13 *

	Plasma						Muscle								
										Fat-free wet muscle					
	Hour	Na mEq./L.	K mEq./L.	Ca mgm. %	P mgm. %	pH	Muscle sample	H_2O %	Fat %	Na mEq./Gm.	K mEq./Gm.	N mgm./Gm.	P mgm./Gm.	Ca mgm./Gm.	Na/K
Dog No. 11	0930	149.8	4.0	12.64	2.6										
	1130	172.4	3.7	9.53	3.9	7.54									
	1300	150.2	2.5	10.25	3.7										
	1530	147.2	2.6	10.35	4.5		Control	63	15.3	.042	.087	29.1	2.02	.065	.5
							Trauma	71	11.8	.103	.031	21.4	1.01	.171	3.3
Dog No. 12	0930	151.4	4.3	12.87	3.6										
	1130	143.5	4.9	12.24	4.5	7.12									
	1300	138.9	5.5	13.70	6.2										
	1530	135.2	6.0	12.02	5.6		Control	70	7.7	.021	.075	27.9	1.71	.082	.3
							Trauma	74	6.0	.078	.045	23.8	1.20	.142	1.7
Dog No. 13	0830	157.2	4.3	11.69	2.1										
	1100	146.6	4.0	11.15	2.9										
	1330	150.2	4.1	10.90	3.3										
	1530						Control	60	13.1	.019	.074	31.9	1.89	.084	.3
							Trauma	60	14.6	.076	.057	26.9	1.29	.123	1.3

* During the six-hour period following trauma and preceding biopsies dog 11 received sodium bicarbonate, dog 12 received hydrochloric acid, and dog 3 received parathyroid extract.

tion between pairs of specimens appeared to be unaffected. The fat content was increased in six and decreased in seven traumatized specimens and appeared to have no significance beyond chance selection of tissue specimens. All traumatized specimens analyzed contained increased sodium, decreased potassium and nitrogen, irrespective of water or fat content. With one exception, phosphate concentration was decreased in all traumatized specimens analyzed, including those from the animals which received phosphate infusions.

DISCUSSION

These experiments demonstrate that the propensity of damaged tissue to accumulate calcium, usually observed radiologically or histologically long after tissue damage, can be detected within a few hours after injury. The calcium in the damaged tissue, presumably bound to denatured protein, does not exchange freely with the calcium in the plasma. In the dog with uremia the quantity of calcium deposited in traumatized muscle is greatly increased, although the calcium content of undamaged muscle is not increased. The peculiarities associated with uremia which provoke these differences are unknown. The administration of phosphate, acid, alkali, and parathyroid extract did not influence the deposition of calcium in muscle under the conditions employed.

The finding of acute calcification of damaged muscle in the uremic dog is consistent with the observation that calcium escapes rapidly from the plasma of patients with post-traumatic uremia. Although the plasma concentration of calcium does not fall in the uremic dog as it does in the uremic man, the average quantity of calcium withdrawn from the plasma is of such a magnitude that one kilogram of damaged muscle would bind the calcium contained in 75 ml. of 10 per cent calcium gluconate (0.67 Gm. calcium). The principal reason for the administration of calcium during post-traumatic uremia is to utilize its antagonistic action toward potassium; 75 ml. of 10 per cent calcium gluconate is a liberal and effective dose for this purpose, and one kilogram of damaged muscle is a conservative estimate of that present in some patients with such severe hyperpotassemia that it must be antagonized immediately to prevent cardiac death. Damaged muscle exaggerates both hyperpotassemia and hypocalcemia, since it releases potassium and accepts calcium. This does not suggest, however, that any direct exchange occurs between these substances of widely different properties. The damaged muscle which releases potassium also accepts sodium, and it is likely that these univalent cations do exchange directly.

The essential factors influencing calcium deposition are unknown, but in uremic man the escape of calcium from plasma is associated with an excess of plasma phosphate, an inorganic anion which may be derived from intracellular phosphorus. Hyperphosphatemia is more acute and extreme in uremic patients with muscle damage, and in the present dog experiments tissue phosphorus, determined as inorganic phosphate, is diminished in damaged muscle. The infusion of phosphate did not increase the deposition of calcium in the damaged dog muscle, although it did provoke hypocalcemia. Hyperphosphatemia and hypocalcemia appear, though more slowly, in anuric patients who have no demonstrable muscle damage, but the precipitation of calcium throughout the body, perhaps in the tissues which released the phosphate, has not been excluded.

Whatever the essential factors controlling calcium deposition may be, it appears possible that the mechanism involves an exchange with a divalent cation. Since it has been observed that plasma magnesium concentration is increased in anuric patients with hypocalcemia (7), a direct exchange between tissue magnesium and plasma calcium is not inconceivable.

SUMMARY

Experiments were performed in dogs to clarify the clinical observation that calcium which is infused into anuric patients escapes more rapidly from the plasma when damaged muscle is present. Using radioactive and standard chemical techniques it was found that damaged dog muscle doubled its calcium content in six hours; the quantity of calcium deposited in damaged muscle was increased by an average of more than tenfold in nephrectomized dogs; and the quantity of calcium contained in undamaged muscle was unaffected by nephrectomy.

These findings are discussed in relation to the administration of calcium for the treatment of potassium intoxication. Speculation on the mechanism of calcification is offered.

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STUDIES ON THE DESTRUCTION OF RED BLOOD CELLS. XII. FACTORS INFLUENCING THE ROLE OF S HEMOGLOBIN IN THE PATHOLOGIC PHYSIOLOGY OF SICKLE CELL ANEMIA AND RELATED DISORDERS [1]

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The deoxygenation of blood from patients with sickle cell anemia leads to transformation of the red blood cells from biconcave discs into the bizarre elongated forms from which the disease derives its name (1). This change in morphology of the red cells is associated with increased viscosity of the blood proportional to the number of red cells so altered (2, 3). Much of the abnormal physiology of sickle cell anemia is attributable to this increase in the viscosity of deoxygenated blood (2–4) as well as to the greater mechanical fragility of the red cells that accompanies the sickled state (5–7). Thus, factors which impede the flow of blood may result in local hypoxia sufficient to increase the extent of sickling. The concomitant increase in viscosity of the blood further decreases blood flow so that local hypoxia becomes more marked, thereby increasing the degree of sickling. In this way the "vicious cycle" of sickle cell anemia (2, 3) is instituted, resulting in localized ischemia and ultimately thrombosis, with necrosis of tissue. The two chief features of sickle cell anemia are thereby explained, the chronic hemolytic anemia on the basis of the increased mechanical fragility and diminished life span of the erythrocytes *in vivo*, and the appearance of painful crises on the basis that various circumstances may temporarily so alter the flow of blood in an organ that the "vicious cycle" of erythrostasis, increased sickling, increased viscosity, etc., occurs and leads to localized ischemia or infarction (2).

Studies of the properties of hemoglobin solutions indicate that the sickling phenomenon is a resultant of the presence of S hemoglobin in the red cells of patients with sickle cell anemia (8, 9) and that the sickled cell may be considered to be a hemoglobin "tactoid" covered by a passively distorted cell membrane. Harris (8) has demonstrated that concentrations of S hemoglobin of at least 10 to 12 grams per cent are required for tactoid formation to occur upon deoxygenation of hemoglobin solutions and that this tactoid formation is associated with increased viscosity of the hemoglobin solutions. Indeed, at concentrations of S hemoglobin of 20 grams per cent or more, deoxygenation leads to gel formation.

These facts and in particular the contrast with the asymptomatic sickle cell trait suggest the desirability of defining in greater detail some of the quantitative aspects of the relationship between S hemoglobin and viscosity changes in whole blood and in solutions of hemoglobin in order that these may be related to physiologic events occurring during the course of the disease. The present study was undertaken in order to add to knowledge of such relationships.

CLINICAL MATERIAL

Twenty-one Negro patients with various hereditary hemoglobinopathies involving S hemoglobin were studied. The pertinent clinical and laboratory data are given in Table I. There were 8 cases of sickle cell anemia (hemoglobin phenotype: S hemoglobin), 9 of sickle cell trait (hemoglobin phenotype: A and S hemoglobins) and 3 of sickle cell-hemoglobin C disease (hemoglobin phenotype: S and C hemoglobins). Finally, in 1 patient 73 per cent of the hemoglobin was S hemoglobin, 21 per cent was A hemoglobin and 6 per cent was F hemoglobin. These findings are not characteristic of sickle cell trait as it is usually defined (10). Morphologically the red cells were normochromic and microcytic even after correction of pre-existing iron deficiency. This hematologic pattern has been called sickle cell-thalassemia disease by Singer, Singer, and Goldberg (11), but inasmuch as the patient's mother was hematologically normal and

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TABLE I

Clinical and laboratory findings in hereditary hemoglobinopathies

Patient	Age	Sex	RBC $\times 10^6$/cu. mm.	HGB Gm. %	HCT %	MCV cu. u	MCHC Gm. %	RETIC %	Icterus index	Per cent hemoglobin S	A	C	F	MCSHC Gm. %	Irreversibly sickled forms %	V_0/V_{∞}*	Painful crises	Red cell half-life *Days*†	Aseptic necrosis of bone
R. W.	25	M	1.96	6.8	19.1	97	35	12.5	20	99	0	0	1.3	35	15.0	3.1	+		0
W. H.	18	M	2.96	8.1	23.0	78	35	8.2	100	96	0	0	3.6	34	16.7	2.8	+		0
Ir. H.	19	F	2.32	6.3	18.0	78	35	11.4	50	91	0	0	9.4	33	7.3	3.1	+		0
In. H.	15	F	1.73	5.6	17.9	103	31	14.1	12	98	0	0	1.7	31	6.4	3.2	+		0
C. Ho.	19	F	3.57	8.2	26.6	75	31	5.5	5	97	0	0	3.0	30	0.4	2.8	+	11	+
R. C.	37	M	3.65	10.0	33.2	91	30	3.7	12.5	95	0	0	4.7	29	3.6	3.3	+		++
E. S.	8	F	2.81	8.1	25.9	92	31	4.8	12.5	89	0	0	11.1	28	7.2	3.0	0		0
B. R.	13	F	3.54	7.7	26.0	73	30	11.2	12.5	90	0	0	10.0	27	6.6	3.2	+		0
B. C.	10	F	2.92	6.2	20.1	69	31	5.2	5	73	21	0	6.4	23	occ.	2.9	+		0
E. B.	30	F	2.93	8.5	24.8	84	34	6.7	5	52	0	47	1.2	18	0.2	2.5	0		0
C. D.	37	F	4.38	10.9	31.8	73	34	0.5	5	50	0	45	5.6	17	0	2.4	0	17	0
L. W.	28	F	4.00	10.8	36.8	92	29		10	52	0	47	0.7	16	1.0	2.4			
W. P.	94	M	3.36	10.6	33.6	100	32	1.7	5	48	51	0	1.5	15	0	1.8	0	22	0
P. H.	6	M	5.28	12.9	40.5	77	32		5	44	56	0	0.5	15	0	1.4	0		0
M. J.	60	F	4.74	13.0	39.7	83	32	2.6	5	45	55	0	0.8	14	0	1.2	0		0
C. Ha.	30	F		12.9	40.1		32	1.2	5	44	54	0	2.3	14	0	1.4	0	30	0
A. B.‡	62	F	2.70	6.7	21.2	79	32	1.5	5	44	55	0	0.9	14	0	1.5	0		0
G. P.	1	M	4.78	10.3	35.1	73	29	2.3	5	45	54	0	1.0	13	0	1.6	0		0
M. G.	23	F	4.79	9.4	32.2	67	29	1.4	5	39	60	0	1.2	11	0	1.2	0		0
B. T.	1	M	3.28	4.5	18.7	57	24	2.4	3	44	55	0	1.2	11	0	1.0	0		0
A. D.	1	F	6.02	10.2	36.2	60	28	0.9	4	35	60	0	4.9	10	0	1.0	0		0
Normal values:		M	5.0±0.3	14.8±0.8	45.9±2.7	91±7	32±2	1.0±0.5	6±2	0	99	0	0.9±0.4	0	0	1.0	0	30–33	0
		F	4.4±0.3	13.0±0.8	42.1±2.0	95±6	31±5												

* Viscosity at pO_2 = 0/Viscosity at pO_2 = 680 mm. Hg.
† Cr^{51} method.
‡ Patient with chronic pyelonephritis and azotemia.

the father was not available for study, the genetic background of the abnormality in this patient could not be confirmed. For convenience, this patient will be designated as having S-A-F disease.

Whole blood will be described according to the contained hemoglobin, so that the designation S-S blood refers to whole blood taken from patients with sickle cell anemia, A-S to blood from patients with sickle cell trait, and so forth.

METHODS

Red blood cell counts, hematocrits, hemoglobin concentrations, mean corpuscular indices, icterus indices, reticulocyte counts and other hematologic determinations were made by standard methods (12). All hematocrit determinations were made on aliquots of whole blood after oxygenation in order to avoid errors introduced by the poor packing of red cells in the sickled form. The presence of the sickling phenomenon was detected by the metabisulfite method (13). The percentage of "irreversibly sickled" red cells (14), or cells which retain their distorted form in the presence of oxygen, was determined by counting the number of such cells per thousand red cells in smears of capillary blood from the ear lobe. The smears were made on cover slips and stained with Wright's stain.

Stroma-free solutions of hemoglobin were prepared by a modification of the method described by Drabkin (15). The modification consisted of using only physiologic saline as the wash fluid for the red cells, and of using the hemoglobin solutions after removal of the stroma by toluene, without crystallization of the hemoglobin. Methemoglobin concentration was measured by the method of Evelyn and Malloy (16), in all hemoglobin solutions, after viscosity measurements had been completed. Because methemoglobins, unlike oxyhemoglobins, do not form tactoids when exposed to low oxygen tensions, viscosity measurements are included in this report only if the percentage of methemoglobin was less than 4 per cent of the total hemoglobin concentration.

Paper electrophoresis of stroma-free hemoglobin solutions was carried out in an apparatus modified slightly from that described by Smith and Conley (17). The paper strips were scanned with a photoelectric densitometer [3] and the area under the resultant curves was measured with a polar planimeter in order to calculate the relative amounts of the various hemoglobins present. The mean corpuscular S hemoglobin concentration (MCSHC) of red cells was calculated by multiplying the mean corpuscular hemoglobin concentration (MCHC) by the percentage of S hemoglobin as obtained from analysis of the paper electrophoretic strips. The methods used for determining MCSHC are reproducible within a range of 5 to 10 per cent. Fetal hemoglobin was measured by the method of Singer, Chernoff, and Singer (18). The percentage of S hemoglobin in the red cells of patients with sickle cell anemia was calculated by subtracting the percentage of fetal hemoglobin present from 100 per cent.

[3] Photovolt densitometer, Model 52 C.

The survival time of labeled red cells in the circulation and sites of their sequestration were determined using radioactive chromium by the methods described by Jandl, Greenberg, Yonemoto, and Castle (19).

The viscosity of whole blood or of hemoglobin solutions was determined according to the methods of Harris, Brewster, Ham, and Castle (3) in Ostwald viscosimeters having bores of about 1 millimeter and flow times for water of 25 to 50 seconds at 37° C. Aliquots of oxalated bloods were equilibrated with gas mixtures containing known percentages of oxygen and nitrogen in the presence of 10 per cent carbon dioxide. Unless otherwise stated the viscosity of samples of whole blood was determined under the following "standard conditions": hematocrit adjusted to 35 per cent when the sample was oxygenated, pH 7.2 to 7.4, and temperature 37° C. maintained in a water bath. The pH of the blood was determined potentiometrically immediately after the flow time had been measured. In some experiments, the pH of whole blood was altered by adding 0.33 M HCl or 0.09 M $NaHCO_3$ prior to equilibration with the gas mixture. It was thus possible to vary the pH of the equilibrated blood from 6.8 to 7.5 without affecting greatly either the hematocrit or the mean corpuscular hemoglobin concentration.

RESULTS

Relation of hemoglobin concentration to viscosity of hemoglobin solutions

The viscosities of solutions of S hemoglobin and of A hemoglobin were determined after complete oxygenation and complete reduction, respectively. The changes in the relative viscosities that occurred in hemoglobin solutions whose concen-

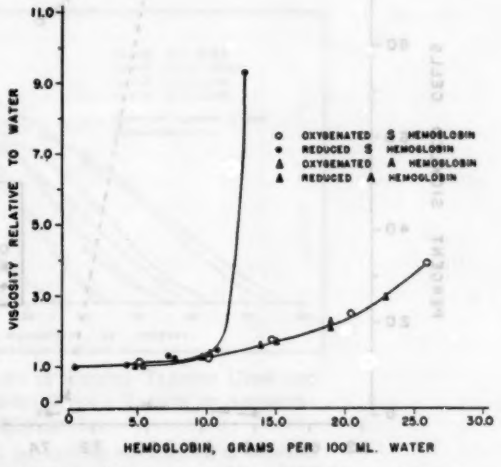

FIG. 1. THE EFFECT OF HEMOGLOBIN CONCENTRATION UPON THE VISCOSITY RELATIVE TO WATER OF STROMA-FREE HEMOGLOBIN SOLUTIONS

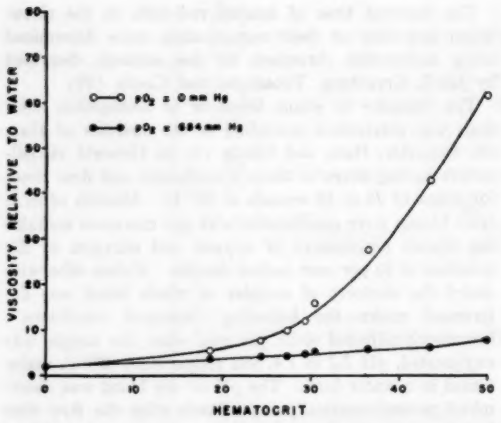

FIG. 2. THE EFFECT OF OXYGEN TENSION UPON THE INCREASE IN VISCOSITY THAT OCCURS WITH INCREASE IN THE HEMATOCRIT OF BLOOD FROM A PATIENT WITH SICKLE CELL ANEMIA

Note that the viscosity of reduced blood at an hematocrit of 25 per cent, an hematocrit frequently found to occur in patients with sickle cell anemia, is about the same as the viscosity of oxygenated blood at an hematocrit of 50 per cent. The curve shown here for oxygenated sickle cell anemia blood is not significantly different from the curve for normal blood. In the case of normal blood there is no difference between the curve for oxygenated blood and that for reduced blood.

trations varied from about 5 to 25 per cent are plotted in Figure 1. The values for reduced A hemoglobin, oxygenated A hemoglobin and oxygenated S hemoglobin fell along a common, gradually ascending curve. The values for reduced S hemoglobin also fell along the same curve for concentrations of less than 10 grams per cent but above this concentration the viscosity of reduced S hemoglobin rose precipitously to levels above those of even the most concentrated of the other hemoglobin solutions. Although not shown in Figure 1 because of the limitations of the scale, the viscosities relative to water of solutions of reduced S hemoglobin in concentrations of 15.2 per cent and 15.9 per cent were 61.2 and 72.2, respectively. At concentrations above 20 grams per cent, the solutions of reduced S hemoglobin were complete gels as observed by Harris (8).

Relation of hematocrit to viscosity of sickle cell anemia blood

Blood from a patient with sickle cell anemia was adjusted to various hematocrit values between 15 and 50 per cent by the addition or removal of autologous plasma. Aliquots of the blood at each hematocrit were fully oxygenated or completely re-

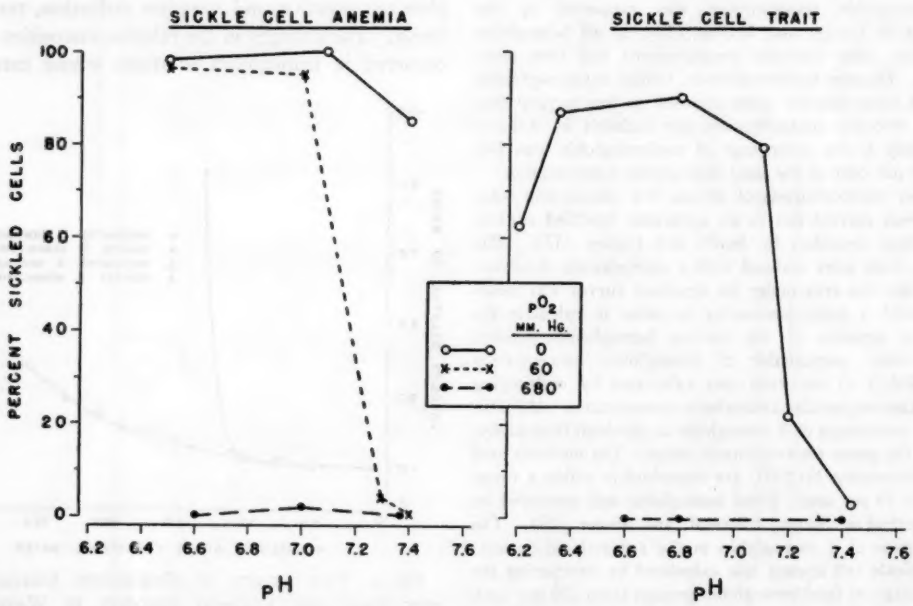

FIG. 3. THE EFFECT OF pH UPON THE SICKLING OF RED CELLS *In Vitro* AT DIFFERENT OXYGEN TENSIONS

duced by equilibration with appropriate gas mixtures. As shown in Figure 2, the viscosity of the oxygenated S-S blood increased slightly as the hematocrit increased. These values correspond with those for normal blood, whether oxygenated or reduced. There is no increase in the viscosity of normal blood upon deoxygenation. However, the viscosity of the reduced S-S blood increased much more rapidly than did that of the oxygenated aliquot especially at hematocrit values above 20 per cent. The figure shows that at a hematocrit of 25 per cent the viscosity of the completely deoxygenated S-S blood was approximately that of oxygenated blood at a hematocrit of 50 per cent.

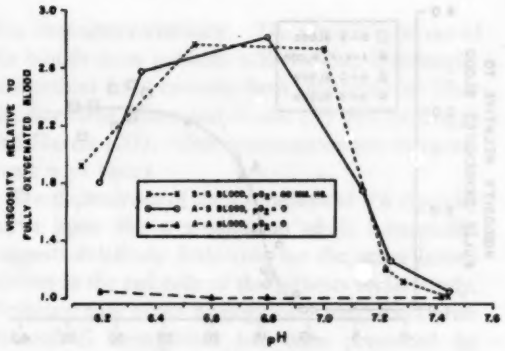

FIG. 4. THE EFFECT OF VARIATIONS IN pH UPON THE VISCOSITY OF WHOLE BLOOD

Effect of pH upon viscosity and the sickling phenomenon

The effect of change in pH upon the degree of sickling and the viscosity of S-S and A-S bloods at several oxygen tensions is shown in Figures 3 and 4. There were no significant effects of pH on viscosity or degree of sickling of S-S or A-S oxygenated blood. However, when the effects of pH were studied at oxygen tensions at which not all of the cells were ordinarily sickled, striking effects on the degree of sickling were observed (Figure 4). Thus, at an oxygen tension of 60 millimeters of mercury the viscosity and degree of sickling of S-S blood increased as the pH was lowered, until a maximum was reached at about pH 6.8 to 7.0. These changes in pH did not cause significant alterations in mean corpuscular hemoglobin concentration. For example, the MCHC was 31 per cent at pH 7.0 when the viscosity was high, and was 32 per cent at pH 7.3 when the viscosity had decreased.

As the pH of A-S blood was lowered to 7.0 at zero oxygen tension the viscosity of the blood approached the maximal values obtained for S-S blood and almost all the cells were sickled. This suggests that A hemoglobin may be incorporated into tactoids at low pH values and oxygen tensions. No effect of pH was observed on the viscosity of fully reduced A-A blood.

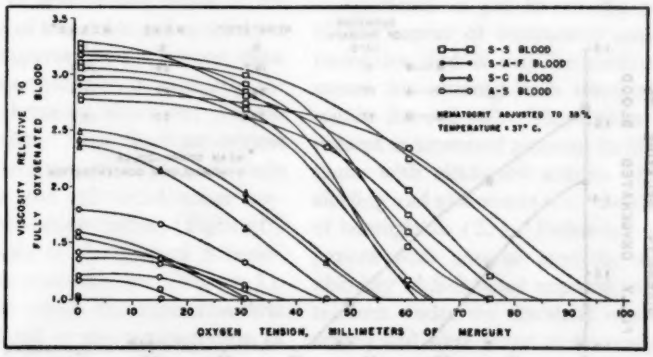

FIG. 5. THE EFFECT OF VARIATIONS IN OXYGEN TENSION UPON THE VISCOSITY OF WHOLE BLOOD OF PATIENTS WITH A VARIETY OF ABNORMAL HEMOGLOBINOPATHIES INVOLVING S HEMOGLOBIN

The hematocrit was adjusted to 35 per cent by the addition or removal of autologous serum in all cases, pH was maintained between 7.2 and 7.3 in all cases by maintaining a pCO_2 of 76 millimeters of mercury, and all observations of viscosity were made on blood at a temperature of 37° C.

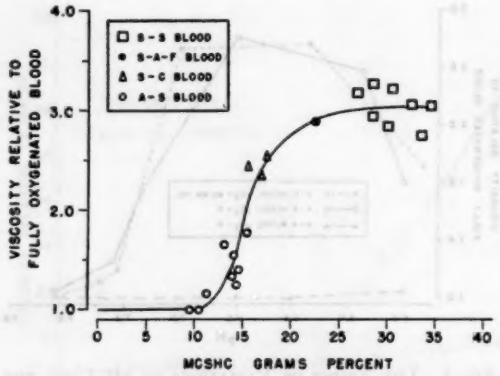

FIG. 6. THE RELATIONSHIP BETWEEN MEAN CORPUSCULAR S HEMOGLOBIN CONCENTRATION (MCSHC) AND THE VISCOSITY OF FULLY DEOXYGENATED BLOOD AT A HEMATOCRIT OF 35 PER CENT

Relation of S hemoglobin concentration within the red cells to the viscosity of whole blood

The alterations in viscosity of oxalated whole blood that occurred at various oxygen tensions under the standard conditions of hematocrit, pH and temperature referred to previously, are illustrated in Figure 5. The curves are based on observations made on the blood of each of the patients described in Table I. The viscosity at complete deoxygenation was greater for S-S blood than for A-S, or S-C bloods, but the viscosity of S-A-F blood fell within the range of S-S bloods. The oxygen tension that barely permitted sickling was also distinctly greater for S-S bloods than for A-S bloods, and was possibly greater than for the other variants of the S hemoglobinopathies. The viscosity curve of S-C blood fell between the curves that characterize S-S and A-S bloods. That of S-A-F blood was among those of the less viscous S-S bloods.

The data in Figure 5 suggest that the mean corpuscular S hemoglobin concentration (MCSHC) and the degree of change in viscosity upon deoxygenation are related phenomena. In Figure 6 is plotted the MCSHC of the red cells of each of the patients described in Table I in relation to the increase in viscosity that occurred when the corresponding blood was deoxygenated completely under the standard conditions of hematocrit, pH, and temperature. The resultant sigmoid curve is comparable to that in Figure 1, in which the viscosity of solutions of various concentrations of S hemoglobin is plotted. Both in the solutions and in the whole blood, and irrespective of the total MCHC, the first increase in viscosity occurred when the MCSHC was greater than 10 grams per cent. Solutions of reduced S hemoglobin became completely gelled at concentrations above 20 grams per cent. Similarly, in whole blood, there was no further increase in viscosity at MCSHC values above about 20 grams per cent, presumably be-

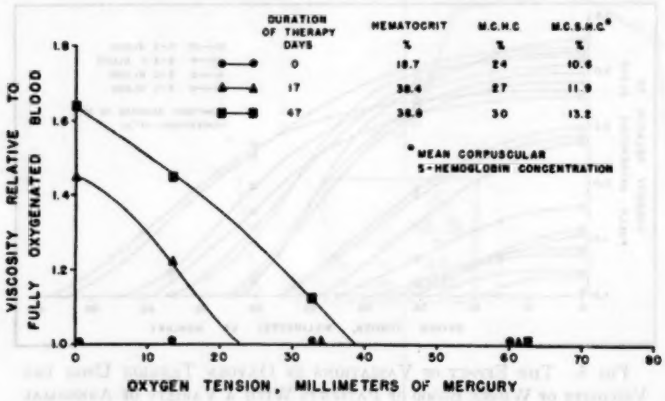

FIG. 7. CHANGES IN THE EFFECT OF DEOXYGENATION UPON THE VISCOSITY *In Vitro* OF BLOOD FROM A PATIENT WITH SICKLE CELL TRAIT (A-S BLOOD) AND COEXISTING IRON DEFICIENCY FOLLOWING TREATMENT OF THE PATIENT WITH FERROUS SULFATE

MCSHC is mean corpuscular S hemoglobin concentration.

cause all the hemoglobin within the red cells was completely gelled.

Further confirmation of the role of MCSHC in determining susceptibility to sickling of red cells came from observations on the bloods of two patients with sickle cell trait and coexisting iron deficiency. The data from one of these patients are presented in Figure 7. Initially, the MCHC was 24 grams per cent and the MCSHC was 10.6 grams per cent. At this time there was no increase in viscosity and no sickling of red cells upon complete deoxygenation of the blood under the standard conditions. However, paper electrophoresis and the metabisulfite tests indicated the presence of S hemoglobin. After iron had been given by mouth for 47 days the ratio of S to A hemoglobin within the red cells did not change, but, as the total MCHC rose, the degree of sickling and the corresponding changes in viscosity approached values commonly seen in sickle cell trait. An increase of MCSHC to 11.9 grams per cent when the MCHC was 27 grams per cent was sufficient to permit sickling after complete dexoygenation; this effect was still greater when the MCHC reached 30 grams per cent and the MCSHC was 13.2 grams per cent.

DISCUSSION

The importance of changes in the viscosity of whole blood *in vivo* in determining much of the pathologic physiology of the sickle cell syndromes (2, 3, 8, 20, 21) is supported by the present data. The S hemoglobin concentration is evidently the chief determinant in bringing about the changes that are observed *in vitro*. Thus, there are evident similarities between the curve of viscosity of whole blood plotted against the calculated mean corpuscular S hemoglobin concentration (Figure 6) and that of the viscosity of solutions of S hemoglobin plotted against concentration (Figure 1). Similarly, the point at which deoxygenation first alters viscosity is related to the concentration of S hemoglobin, whether in solution or in cells.

When the viscosity curves of the bloods of patients with various S hemoglobinopathies are compared, a similar relationship is seen between the mean corpuscular S hemoglobin concentrations and both the degree of viscosity change upon deoxygenation and the point at which deoxygenation first alters viscosity. The viscosity curves of the bloods from patients with various S hemoglobinopathies have recently been described by Harris, Brewster, Ham, and Castle (3) and by Griggs and Harris (20). Our observations are in agreement with theirs.

The dependence of the alterations of the viscosity curve upon the concentration of S hemoglobin suggests relatively little role for the other hemoglobins in the red cells of the patients under study. Evidence that other hemoglobins may react with reduced S hemoglobin has been presented by Singer and Singer (21). In the present observations the interaction of other hemoglobins with S hemoglobin was greatest at pH values lower than the usual physiologic range, as shown by the increased viscosity of fully reduced A-S blood as the pH was lowered below 7.4 (Figure 4). On the other hand, the viscosity changes that occurred in reduced A-S and S-C bloods at physiologic and higher pH values fell in the range that would be anticipated from the S hemoglobin concentrations alone. Thus, the interaction of A and C hemoglobins with S hemoglobin is probably minimal at normal physiologic pH values, but may play a role if intracellular pH is lowered. Conversely, the interaction of reduced hemoglobin molecules is diminished at pH values above 7.4 and even S-S interaction appears to be decreased slightly under such circumstances.

Alteration in pH is not the sole determinant of the extent of interaction among hemoglobin molecules, and it may be anticipated that other means for altering such interaction are present within the red cell. Thus, glutathione, which is present in increased amounts in the red cells of patients with sickle cell anemia (22) itself causes sickling and also reacts with the sulfhydryl groups of hemoglobin (23). Following treatment of the patient with adrenal steroids, decreases in the viscosity of S-S blood exposed to reduced oxygen tensions under the standard conditions were observed and were found to be associated with lowered blood glutathione levels (22). Similarly, there is evidence that there is an optimal concentration of potassium ions for sickling to occur under physiologic conditions (24).

The requirement of a mean corpuscular S hemoglobin concentration of approximately 10 grams per cent before sickling appears in deoxygenated

blood under standard conditions indicates the lower limit of sensitivity of the deoxygenation test for the sickling phenomenon. Thus, the blood of the patients with sickle cell trait and iron deficiency anemia did not sickle after deoxygenation until the MCSHC had increased to more than 10 grams per cent following the administration of iron (Figure 7). However, sickling of the bloods of the patients with iron deficiency was observed in the presence of metabisulfite even when the MCSHC was insufficient to permit sickling upon deoxygenation. The greater sensitivity of the metabisulfite test may be ascribed to the lowered pH at which the test is performed and perhaps to the possibility that strong reducing agents may increase the degree of interaction of hemoglobin molecules beyond that observed with deoxygenation alone. We have observed that when the MCSHC is reduced by suspending A-S cells in hypotonic solutions, with resulting dilution of cell contents by the entry of water, the lower limit of S hemoglobin concentration in the red cells that permits sickling with metabisulfite is about 7 grams per cent.

The determination of the limits of sensitivity of the usual screening tests for the presence of the sickling phenomenon poses certain problems. Since bloods with MCSHC values of less than 7 grams per cent do not sickle in the metabisulfite test and since this concentration of S hemoglobin approximates one-fourth to one-fifth of the total hemoglobin in a red cell, it follows that when S hemoglobin accounts for less than 20 to 25 per cent of the hemoglobin present in the fully hemoglobinated cell, the cells will not sickle. Hence it is not surprising that surveys of the ratios of S to A hemoglobins in patients with sickle cell trait have not uncovered instances in which less than 20 per cent of the hemoglobin was S hemoglobin (10). Yet, there is no apparent reason why individuals may not carry lower concentrations of S hemoglobin in their bloods. Such an eventuality would remain undetected because of the limitations of the sickling tests and the paper electrophoretic method as usually conducted. For example, Singer and Fisher (25) have shown by careful electrophoretic study of the blood of one patient whose blood did not sickle, that only 5 per cent of the total hemoglobin was S hemoglobin (MCSHC, 1.7 grams per cent). It may be anticipated that more sensitive methods will increase the number of instances in which S hemoglobin in amounts lower than 20 per cent of the total will be identified in the red cells of patients whose blood fails to sickle with metabisulfite. Conceivably, the present methods of detecting sickling can be modified to increase the likelihood of hemoglobin interaction, and hence to increase the sensitivity of such tests.

There is little difference between the viscosity of completely deoxygenated S-S blood at a hematocrit of 25 per cent and that of fully oxygenated blood at a hematocrit of 50 per cent (Figure 2). Thus, the physiologic consequences of sickling *in vivo* are presumably small except at sites of erythroconcentration. The spleen is such a site (2, 26); it is frequently enlarged, and may be a locus of distress in children with sickle cell anemia (27). The small fibrotic spleen characteristic of adult patients with sickle cell anemia is undoubtedly the end result of repeated episodes of splenic congestion and infarction. Again, patients with sickle cell trait or sickle cell-hemoglobin C disease, who are generally asymptomatic, may develop splenic infarction as a consequence of the hypoxia that may occur during travel at high altitudes (28). Similar adverse effects are to be anticipated as a consequence of hypoxia or of increased carbon dioxide concentrations in the blood, such as may occur during anesthesia.

The auto-splenectomy that occurs in adults with sickle cell anemia may act to the patient's advantage. Thus, the red cells of patient Co. Ho., whose spleen was not palpable, had a half-life of 11 days in the patient herself by the Cr^{51} method (19). The patient's labeled red cells tended to be sequestered in the liver, a site of low pressure blood flow, with no apparent concentration in the area of the spleen. However, when her labeled red cells were transfused into a normal recipient with an intact spleen, the half-life of her cells was only 6 days. In this recipient radioactivity appeared predominantly over the area of the spleen as well as that of the liver (19).

The clinical manifestations of the presence of S hemoglobin are related to the likelihood of sickling under physiologic oxygen tensions, which, in turn, appears to be dependent upon a sufficient intracellular S hemoglobin concentration. Thus, anemia, painful crises, and destructive bone

changes, such as aseptic necrosis of the head of the femur, occurred in our patients only in those with sickle cell anemia in whom the MCSHC was 28 per cent or higher. The patient with S-A-F disease in whom the MCSHC was 23 per cent also had both anemia and painful crises. No lesions of the bones were present but the patient is only 10 years old. Such lesions have been reported in other patients with S-A-F disease (29). Smith and Conley (30) have noted aseptic necrosis of the head of the femur in patients with sickle cell-hemoglobin C disease, but data permitting quantitation of the MCSHC in their patients were not published.

Mild anemia, without painful crises or bone lesions, was noted in our 3 patients with sickle cell-hemoglobin C disease, in whom the MCSHC was 16 to 18 per cent. In addition, the half-life of the red cells of one of these patients was found to be 17 days by the Cr^{51} method, which is appreciably shorter than the normal range of 30 to 33 days for this method. Similarly, one patient with sickle cell trait and an MCSHC of 15 grams per cent evidenced a moderate decrease in the red cell survival time, the half-life being 22 days. Other patients with sickle cell trait were not anemic unless a complication was present, such as iron deficiency (patients A.D. and B. T.) or uremia (patient A.B.) as noted in Table I.

One patient (C. Ha.) with sickle cell trait and an MCSHC of 14.1 grams per cent was observed during a severe attack of lobar pneumonia to have developed mild anemia and increased susceptibility to sickling of her red cells at physiologic oxygen tensions capable of initiating sickling of S-S blood. However, after recovery, the susceptibility of her red cells to sickling at such oxygen tensions diminished and their survival time *in vivo* became normal. The subsequent administration of cortisone failed to induce an increase in the susceptibility to sickling of her red cells.

The increased viscosity that occurs when red cells have been deoxygenated under standard conditions of hematocrit, pH and temperature supplies a simple method for quantitating the susceptibility to sickling of red cells. Many uses may be suggested for this technique. The viscosity responses may provide a means for following the altered susceptibility to sickling of red cells during treatment, as has been done here in patients with iron deficiency, or as has been done in studies of the effects of adrenocortical hormones in patients with sickle cell anemia (22). The viscosity curves have also been useful in patients with sickle cell anemia who have received transfusions of normal red cells. Under these circumstances many normal red cells are in the circulation and paper electrophoretic patterns of the hemolyzed blood may show relatively large amounts of A hemoglobin. However, because the patient's own red cells contain a high concentration of S hemoglobin, the viscosity of the blood under the standard conditions begins to increase at oxygen tensions above 40 millimeters of mercury, a finding that has not been observed in sickle cell trait. On the other hand, because the normal transfused cells do not sickle, the maximum viscosity of the blood under the standard conditions is not as great as that of the blood before transfusion. Finally, the viscosity curves are of value in following patients who develop painful crises. During a crisis and for variable periods of time thereafter, the blood of patients with S hemoglobin syndromes may evidence increased capacity to sickle under standard conditions at oxygen tensions intermediate between complete oxygenation and complete reduction (31).

Since this increased susceptibility to sickling of red cells can be reproduced simply by lowering the pH of the blood, it follows that acidosis may increase the degree of sickling *in vivo* and that alkalosis may reduce the magnitude of such changes. Indeed, it has been possible to induce painful crises in a patient with sickle cell anemia by administering acidifying agents, and to terminate the crises so produced, as well as spontaneous crises, by the administration of alkali (32).

SUMMARY

Twenty-one patients with various hereditary hemoglobinopathies involving S hemoglobin were studied with respect to the concentration of the different hemoglobins in their red cells, the clinical manifestations of the sickling phenomenon, and the changes in viscosity of their bloods upon deoxygenation *in vitro* under standard conditions of hematocrit, carbon dioxide tension and temperature.

A general relationship was demonstrated between the mean corpuscular S hemoglobin con-

centration (MCSHC) and the clinical manifestations of the S hemoglobinopathies. Patients in whom the MCSHC was less than 15 grams per cent were not anemic and had no painful crises. Those in whom the MCSHC was 15 to 18 grams per cent had evidence only of a mild hemolytic anemia. Significant anemia, bony lesions, and painful crises were limited to patients with MCSHC values of more than 20 grams per cent.

The effect of progressive deoxygenation upon the viscosity of whole blood at standard hematocrit and pH was also a function of MCSHC. In the pH range 7.2 to 7.4, the changes in viscosity upon deoxygenation were similar to those of solutions of S hemoglobin and viscosities rose sharply as the concentrations of S hemoglobin increased above 10 grams per cent. Similarly, less complete deoxygenation was necessary to elicit the first changes in viscosity at higher than at lower S hemoglobin concentrations.

Viscosity changes in bloods from patients with S hemoglobinopathies were also related to pH. Decreasing the pH of such bloods when partially deoxygenated led to increases in viscosity maximal at about pH 6.8 to 7.0. Conversely, raising the pH decreased the viscosity of partially deoxygenated blood. However, decreasing the pH of fully reduced S-S blood did not significantly increase its viscosity, whereas with fully reduced S-A blood the effect was striking. The evidence suggests that at pH values of 7.2 to 7.4, the hemoglobin concentrations are the major determinants of the changes in viscosity upon deoxygenation, but that at lower pH values S hemoglobin interacts with other hemoglobins.

The observations are consistent with the previously expressed concepts that the increased viscosity of blood that occurs upon deoxygenation is a major factor in the pathophysiology of the S hemoglobinopathies. From the *in vitro* data it may be inferred that the changes in viscosity are greatest at sites of erythroconcentration, and are augmented by lowering the pH and diminished by raising the pH of the blood.

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HEMODYNAMIC EFFECTS OF QUINIDINE: INCLUDING STUDIES OF CARDIAC WORK AND CORONARY BLOOD FLOW [1]

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There have been many observations concerning the hemodynamic and electrocardiographic effects of quinidine, both in the experimental animal and in man (1). Although quinidine produces rather profound changes in metabolism at the cellular (2, 3) and subcellular (4) level, as well as in the body as a whole, (5), there has been relatively little demonstrable change hemodynamically in normal patients receiving therapeutic doses of the drug (6–8). It appeared that *in vivo* myocardial metabolic data might be of interest in the study of a dose of quinidine previously shown to be effective in preventing cyclopropane epinephrine arrhythmia in the dog (9). Further justification for studying a dose thus selected is the known poor correlation between blood and tissue quinidine levels (10) which precludes administration of a quantity calculated to reproduce the levels studied *in vitro*. The data include systemic hemodynamics along with coronary blood flow, myocardial oxygen consumption, and cardiac efficiency.

MATERIAL AND METHODS

A total of 16 dogs weighing between 16 and 32 kilograms was studied in the post-absorptive state. Cardiac outputs were determined by the Fick principle and coronary blood flows by the nitrous oxide method. The procedure used has been described previously from this laboratory and was similar in this study except that mixed venous blood was withdrawn from the pulmonary artery in all but Dogs Nos. 2 and 3, where the right ventricular blood was utilized as before (11). The plan of anesthesia was 3 mg. per kilogram of morphine intramuscularly followed in one hour by 12 mg. per kilogram of sodium pentobarbital intravenously. The control observations were begun approximately 1 hour after the pentobarbital was given.

In the first 11 dogs, 15 mg. of quinidine gluconate per kilogram were given intravenously over a two-minute period. The second cardiac output was begun approximately 25 minutes after the drug was given and followed immediately by the second determination of coronary blood flow. Because the rapid administration of quinidine produced a very severe hypotension, the last five dogs were given quinidine slowly over twenty minutes and the second observations were begun within two or three minutes after the end of the injection of quinidine. Since the results appeared to be independent of the method of administration, both groups were summarized together.

On two occasions, it was necessary to modify the plan of anesthesia since inadequate anesthesia was obtained and further sedation was required. The first, Dog No. 4, was discarded because large doses of pentobarbital were required prior to the first and second hemodynamic determinations. The other, Dog No. 7, had supplemental morphine and pentobarbital before the first study and remained basal throughout. One further study, Dog No. 6, was discarded from the series because during the placement of cardiac catheters, supraventricular tachycardia occurred with a control rate of 287 beats per minute. Data from Dogs Nos. 4 and 6 are included at the bottom of Table I and excluded from the calculations. In Dog No. 16, data concerning cardiac output and coronary flow were not obtained. All dogs accepted in the series had sinus rhythm except Dog No. 3, which had auricular fibrillation during the first study with a slow rate which converted to sinus rhythm after administration of quinidine.

RESULTS

Immediately upon administration of the quinidine, whether given rapidly or slowly, there was an acceleration of cardiac rate. This acceleration persisted for as long as the animals were observed and, at the time of the second set of determinations, was increased by 98 per cent ($p < 0.001$). Femoral arterial blood pressure fell with administration of quinidine, particularly when it was given rapidly. The greatest hypotension was reached shortly after the termination of the injection of

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quinidine and pressure then rose again until at the time of the second determinations it averaged 7 per cent lower than the initial observations, which did not represent a significant change ($p < 0.1$). Immediate changes in the pulmonary arterial blood pressure were not as marked as those in the systemic vessels. However, at the time of the second determinations there was a consistent lowering of pulmonary arterial pressure (-29 per cent, $p < 0.001$). The minute volume of respiration increased immediately after administration of quinidine and remained elevated at the time of the second cardiac output ($+37$ per cent, $p < 0.001$). Oxygen consumption and CO_2 liberation increased in every case, the former by 12 per cent ($p < 0.001$) and the latter by 21 per cent ($p < 0.001$). Hence there was a significant decrease in exchange of respiratory gases per liter of ventilation ($p < 0.001$).

Although the arterial oxygen content was not changed significantly, the mixed venous O_2 content fell (-7 per cent, $p < 0.01$) resulting in a significant increase in the arterio-venous blood oxygen difference ($+21$ per cent, $p < 0.001$). A significant decrease in coronary sinus blood oxygen (-40 per cent, $p < 0.001$) was noted as well as an increase in arterial-coronary sinus venous oxygen difference ($+19$ per cent, $p < 0.001$).

There was no significant change in cardiac output (-3 per cent, $p < 0.9$). Stroke volume, however, fell rather markedly due to the tachycardia (-50 per cent, $p < 0.001$). Total peripheral resistance was unchanged but there was a decrease in total pulmonary vascular resistance in every experiment which was significant (-23 per cent, $p < 0.001$). As a manifestation of the unchanged cardiac output and peripheral resistance, left ventricular work was not significantly altered (-9 per cent, $p < 0.1$). Right ventricular work, on the other hand, was significantly decreased (-33 per cent, $p < 0.001$).

Coronary blood flow was significantly increased ($+46$ per cent, $p < 0.001$) and since this was accompanied by an increase in arterial-coronary sinus blood oxygen difference ($+19$ per cent, $p < 0.001$), the cardiac metabolic rate for oxygen was markedly increased ($+77$ per cent, $p < 0.001$). If the left ventricular work be divided by oxygen consumption per 100 Gm. of myocardium per minute, a figure is obtained which has been called in this study the index of efficiency. This figure is clearly related to the efficiency of the heart, yet avoids estimation of cardiac weight. This index reveals a 45 per cent decrease in myocardial efficiency ($p < 0.001$) after quinidine administration. If calculations be done, assuming the left ventricular weight to be 0.0037 times the body weight, the average cardiac efficiency was 31 per cent before and 16 per cent after quinidine administration.

Examination of the electrocardiogram on these animals was somewhat unsatisfactory. It was especially difficult to determine changes in T waves and QT interval, because the acceleration was so great during the second set of determinations that the P waves and T waves ran together, making it impossible to measure intervals accurately.

Observations concerning the correlation between each of the factors measured in this study were calculated in the numerical analysis laboratory of the University of Wisconsin and surveyed for significant correlations. Correlation coefficients were determined for every function measured with every other function, in the control data and in the data obtained after quinidine. This was done to attempt to determine what correlations existed in the control animals and how they were modified, if at all, by the drug. These correlations have been summarized in Table II. Only those correlations which were thought to be of interest are included. As indicated in the table, some of those factors which have the highest "r" values owe this, at least partially, to a common factor used in their calculation. It should be emphasized that for this and other reasons, mathematical correlation does not necessarily signify physiological correlation.

DISCUSSION

Quinidine intravenously in the dosage of 15 mg. per kg. was followed by tachycardia associated with a marked increase in myocardial oxygen consumption without any significant change in left ventricular work. Of particular interest was the fact that the increase in myocardial oxygen consumption was associated with an increase in coronary blood flow and an increase in arterial-coronary sinus blood oxygen difference. This is in contrast to previous studies in which an increase in coronary flow was accompanied by either no significant change in arterial-coronary sinus oxygen difference (12) or a decrease (13).

TABLE

Study of quinidin

Study date & Number	Study	Wt. Kg.	Rate	Syst.[3] MABP	PA[4] MABP	Min.[5] vol. resp.	O_2[6] cons.	O_2[7] Cons./ L. vent.	CO_2[8] exc.	CO_2[9] exc./ L. vent.	R.Q.[10]	Art.[11] O_2	M. Ven.[12] O_2	Δ^{13} A-M Ven. O_2	M. Ven.[14] CO_2	Art.[15] CO_2	Δ M.[16] Ven.-A CO_2	CS[17] O_2	Δ^{18} A-CS O_2
12/15/55	C[1]	21	96	120		3.6	122	36	94	26	0.77	20.1	15.8	4.3	51.6	48.6	3.2	6.9	13.2
No. 2	D[2]		135	128		4.3	132	30	102	24	0.77	19.3	13.5	5.7	51.5	47.3	4.2	3.9	15.4
12/20/55	C	20	72	73		2.2	80	31	71	32	0.80	11.8	8.5	3.3	58.2	55.2	3.1	6.9	4.9
No. 3	D		101	91		3.2	98	31	80	25	0.82	12.0	7.6	4.5	54.7	51.8	3.0	5.0	7.0
1/9/56	C	16	97	97	10	1.5	87	57	61	40	0.70	17.4	14.1	3.3	51.5	49.0	2.5	6.0	12.2
No. 5	D		189	91	7	2.5	104	42	84	34	0.81	17.8	13.0	4.8	47.3	44.5	2.8	4.0	15.5
1/19/56	C	25	111	112	18	3.1	130	45	92	30	0.66	16.1	11.0	5.1	55.3	52.3	3.0	4.9	11.5
No. 7	D		176	106	14	5.0	152	31	119	24	0.78	16.2	10.6	5.6	50.5	46.4	4.1	2.5	13.7
1/23/56	C	21	86	112	16	2.3	100	43	80	35	0.80	19.6	17.6	2.0	54.9	53.0	1.9	10.3	9.4
No. 8	D		117	120	12	2.7	103	38	85	32	0.83	19.4	17.2	2.2	52.0	50.6	1.5	4.5	14.7
1/25/56	C	17	78	109	12	3.6	108	30	84	24	0.78	19.5	16.1	3.4	45.9	43.4	2.5	6.2	13.6
No. 9	D		140	107	6	3.8	106	28	86	23	0.81	18.8	15.1	3.7	43.5	41.0	2.5	3.3	15.4
1/30/56	C	20	75	103	19	2.8	118	42	87	31	0.74	17.8	13.4	4.4	47.2	44.1	3.1	6.1	12.0
No. 10	D		192	69	15	4.8	135	28	112	23	0.83	18.0	12.7	5.3	39.9	35.5	4.4	4.4	14.8
2/1/56	C	21	89	127	9	2.7	103	38	83	31	0.81	19.5	15.6	3.9	56.7	53.9	2.8	4.9	14.7
No. 11	D		192	113	8	3.3	113	35	95	29	0.84	20.5	16.5	4.0	53.2	50.5	2.7	3.6	17.1
2/8/56	C	21	68	98	10	2.7	123	45	96	35	0.78	14.6	10.0	4.6	53.3	49.8	3.5	4.0	11.0
No. 12	D		160	96	9	3.9	149	38	124	32	0.83	14.3	10.0	4.3	48.7	45.5	3.2	2.6	12.7
																			Slow Injection Ove
2/8/56	C	20	89	109	15	2.0	75	38	60	31	0.80	16.0	11.7	4.3	48.3	44.8	3.5	3.8	13.4
No. 13	D		160	90	11	3.0	82	28	71	24	0.87	14.4	9.2	5.2	46.9	42.3	4.6	1.8	13.2
2/9/56	C	20	81	135	15	2.4	107	44	78	32	0.73	16.8	12.6	4.2	56.2	53.5	2.7	3.6	13.7
No. 14	D		205	110	10	2.9	115	40	92	32	0.80	16.5	11.2	5.3	54.0	50.3	3.7	2.1	15.1
2/13/56	C	24	89	122	13	3.1	114	37	88	29	0.77	14.8	11.0	3.8	55.8	53.4	2.4	3.9	11.4
No. 15	D		162	113	9	4.5	131	29	115	26	0.88	15.2	10.2	5.0	55.4	51.7	4.7	2.4	12.9
2/14/56	C	22	72	109	10							20.0	15.2	4.8	46.0	42.0	4.0	7.1	13.2
No. 16	D		128	82	8							18.7	12.1	6.6	45.7	41.7	4.0	3.1	15.5
2/15/56	C	22	60	89	15	3.1	106	34	85	27	0.80	19.5	16.3	3.2	52.9	51.6	1.3	9.1	10.8
No. 17	D		176	83	12	4.5	134	30	110	25	0.82	19.3	16.1	3.2	49.1	47.3	1.8	5.2	14.3
Total before		290	1,133	1,515	162	35.1	1,391	520	1,059	403	9.94	243.5	188.9	54.6	7,340	694.6	39.5	83.7	165.0
Ave. before		21	81	108	14	2.7	107	40	81	31	0.76	17.4	13.5	3.9	52.4	49.6	2.8	6.0	11.8
St. Dev.			±15	±16	±3.4	±0.6	±17	±7	±11	±4	±0.05	±2.5	±2.8	±0.8	±4.1	±4.4	±0.7	±2.1	±2.4
Total after			2,233	1,390	121	48.4	1,554	428	1,275	353	10.64	240.4	175.0	65.4	6,924	646.4	47.2	48.4	197.3
Ave. after			160	100	10	3.7	120	33	98	27	0.82	17.2	12.5	4.7	49.5	46.1	3.4	3.5	14.1
St. Dev.			±31	±17	±3.0	±0.8	±21	±5	±17	±4	±0.03	±2.5	±2.9	±1.1	±4.5	±4.8	±1.0	±1.1	±2.4
Difference			+79	−8	−4	+1.0	+13	−7	+17	−4	+0.06	−0.2	−1.0	+0.8	−2.9	−3.5	+0.6	−2.5	+2.3
% Change			+98%	−7%	−29%	+37%	+12%	−18%	+21%	−13%	+8%	−1%	−7%	+21%	−6%	−7%	+21%	−40%	+19%
p-Value			<0.001	<0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.4	<0.01	<0.001	<0.001	<0.001	<0.02	<0.001	<0.001
1/4/56	C	32	112	132	16	4.5	172	38	123	28	0.72	18.6	13.3	3.3	52.8	49.0	3.8	6.8	10.7
No. 4	D		133	117	15	5.0	186	37	125	25	0.67	16.6	13.3	3.3	49.6	46.7	2.9	4.1	12.7
1/16/56	C	32	287	98	19	4.6	144	31	138	30	0.96	20.7	14.2	6.5	47.4	42.5	4.9	3.7	16.1
No. 6	D		219	91	12	5.3	187	35	147	28	0.79	18.1	13.3	4.8	47.0	43.2	3.8	3.0	15.0

1. Control Study.
2. Study after quinidine 15 mg. per kg. intravenously.
3. Femoral arterial mean blood pressure in mm. Hg.
4. Pulmonary arterial mean blood pressure in mm. Hg.
5. Minute volume of respiration in liters per minute.
6. Oxygen consumption in ml. per minute.
7. Oxygen consumption in ml. per liter of ventilation.
8. Carbon dioxide eliminated in ml. per minute.
9. Carbon dioxide eliminated in ml. per liter of ventilation.
10. Body respiratory quotient.
11. Femoral arterial oxygen content in ml. per 100 ml. of blood.
12. Pulmonary arterial (mixed venous) oxygen content in ml. per 100 ml. of blood.
13. Arterio-venous oxygen difference in ml. per 100 ml. of blood.
14. Pulmonary arterial CO_2 content in ml. per 100 ml. of blood.
15. Femoral arterial CO_2 content in ml. per 100 ml. of blood.
16. Mixed venous-arterial CO_2 difference in ml. per 100 ml. of blood.
17. Coronary sinus blood oxygen content in ml. per 100 ml. of blood.
18. Arterial-coronary sinus blood oxygen difference in ml. per 100 ml. of blood.
19. Coronary sinus blood CO_2 content in ml. per 100 ml. of blood.
20. Coronary sinus-arterial CO_2 content difference in ml. per 100 ml. of blood.

ABLE I

inidine with dogs

[18] CS O_2	CS[19] CO_2	Δ[20] CS-A CO_2	Card.[21] R.Q.	C.O.[22] L/M	Tot.[23] peri. res.	Tot.[24] pulm. res.	L.V.[25] work	R.V.[26] work	Cor.[27] blood flow	CMR[28] O_2	CMR[29] CO_2	CVR[30]	St.[31] vol.	Art.[32] hgb.	Art.[33] het.	C.B.F.[34]/ beat	C.B.F.[35] *Kg.* L.V.W.	Ind. eff.[36] L.V.W./ $CMRO_2$	Time[37]	Dose[38]
13.2	58.8	10.2	0.78	2.9	3,309		4.9		82	10.9	8.4	1.5	29	16.6	50	0.86	16.7	0.45	24	315
15.4	57.7	10.4	0.68	2.3	4,427		4.2		93	14.3	9.7	1.4	17	15.5	47	0.68	21.6	0.29		
4.9	58.0	3.8	0.78	2.7	2,193		2.8		96	4.7	3.6	0.8	37	9.8	31	1.34	34.3	0.60		
7.0	55.9	5.2	0.74	2.2	3,305		3.2		124	8.7	6.4	0.7	22	9.6	31	1.22	38.8	0.37	15	300
12.2	57.1	9.4	0.77	2.6	2,943	303	3.5	0.4	70	8.5	6.6	1.4	27	16.4	49	0.72	20.0	0.41		
15.5	51.2	11.5	0.74	2.2	3,358	258	2.7	0.2	100	15.5	11.5	0.9	12	15.7	46	0.53	37.0	0.17	28	240
11.5	59.6	9.1	0.79	2.7	3,282	528	4.2	0.7	60	6.9	5.5	1.9	24	14.9	42	0.54	14.2	0.61		
13.7	54.6	9.3	0.69	2.7	3,122	412	3.9	0.5	103	14.1	9.6	1.0	15	14.1	41	0.38	20.4	0.38	27	375
9.4	59.8	7.5	0.80	5.0	1,788	255	7.6	1.1	100	9.4	7.5	1.1	58	15.5	45	1.16	13.2	0.81		
14.7	59.3	10.2	0.70	4.7	2,057	204	7.7	0.8	135	19.8	13.8	0.9	40	15.2	45	1.15	17.5	0.56	24	315
13.6	51.7	9.2	0.68	3.2	2,743	302	4.7	0.5	82	11.2	7.5	1.3	41	16.7	48	1.04	17.4	0.42		
15.4	50.7	11.2	0.73	2.9	2,985	167	4.2	0.2	89	13.7	10.0	1.2	21	15.0	45	0.64	21.2	0.31	26	255
12.0	52.5	8.3	0.69	2.7	3,069	566	3.8	0.7	82	9.8	6.8	1.3	35	15.6	47	1.09	21.6	0.39		
14.8	45.1	10.5	0.71	2.5	2,165	471	2.4	0.5	111	16.4	11.7	0.6	13	16.3	51	0.88	46.3	0.15	25	340
14.7	66.5	13.4	0.91	2.6	3,843	272	4.6	0.3	56	8.2	7.6	2.3	29	16.1	47	0.63	12.2	0.56		
17.1	62.4	13.4	0.78	2.8	3,107	226	4.3	0.3	103	17.6	15.2	1.1	15	17.0	50	0.54	24.0	0.28	31	308
11.0	56.9	7.7	0.70	2.7	2,884	294	3.6	0.4	84	9.1	6.5	1.2	40	12.8	39	1.24	23.4	0.40		
12.7	54.0	8.7	0.69	3.5	2,214	208	4.5	0.4	110	14.0	7.9	0.9	22	12.9	39	0.69	24.5	0.32	30	306
ion Over 20 Minutes																				
13.4	53.5	11.4	0.85	1.7	4,994	687	2.6	0.4	37	5.0	4.2	3.0	29	14.0	42	0.63	14.2	0.52		
13.2	51.4	10.7	0.81	1.6	4,562	558	1.9	0.2	68	9.0	7.3	1.3	10	13.3	40	0.43	35.8	0.21	23	308
13.7	61.9	8.8	0.64	2.5	4,235	470	4.7	0.5	84	11.5	7.4	1.6	31	14.9	44	1.04	19.7	0.41		
15.1	58.7	10.3	0.68	2.2	4,051	368	3.2	0.3	133	20.1	13.7	0.8	11	14.3	42	0.65	41.6	0.16	20	300
11.4	63.3	9.4	0.82	3.0	3,250	349	5.0	0.5	68	9.0	6.4	1.8	34	12.7	36	0.76	13.6	0.64		
12.9	60.5	9.5	0.74	2.6	3,405	271	4.1	0.3	122	18.9	11.6	0.9	16	12.7	37	0.75	29.8	0.26	22	354
13.2	51.9	10.4	0.79											16.9	51					
15.5	51.3	11.3	0.73											15.9	47				23	330
10.8	59.3	8.2	0.76	3.3	2,147	362	4.0	0.7	92	9.9	7.5	1.0	55	16.7	47	1.53	23.0	0.40		
14.3	55.6	9.9	0.69	4.2	1,598	220	4.7	0.7	148	21.2	14.6	0.6	24	16.2	46	0.84	31.5	0.22	29	338
35.0	810.8	126.8	10.76	37.6	40,680	4,388	56.0	6.2	993	114.1	85.5	20.2	470	209.6	618	12.58	243.5	6.62	340	4,374
11.8	57.9	9.1	0.77	2.9	3,129	399	4.3	0.6	76	8.8	6.6	1.6	36	15.0	44	0.97	18.7	0.51	24	312
±2.4	±4.4	±2.2	±0.07	±0.7	±874	±142	±1.3	±0.2	±18	±2.2	±1.4	±0.6	±10	±2.0	±6	±0.30	±6.1	±0.13		
97.3	768.4	142.1	10.11	36.4	40,446	3,372	51.0	4.4	1,439	203.3	143.0	12.3	238	203.7	607	9.28	396.0	3.58		
14.1	54.9	10.2	0.72	2.8	3,111	307	3.9	0.4	111	15.6	11.0	0.9	18	14.6	43	0.71	30.5	0.28		
±2.4	±4.7	±1.8	±0.04	±0.9	±915	±127	±1.4	±0.2	±21	±3.9	±2.8	±0.2	±8	±2.0	±5	±0.23	±8.9	±0.11		
-2.3	−3.0	+1.1	−0.5	−0.1	−18	−92	0.4	−0.2	35	6.8	+4.4	0.7	−18	−0.4	−1.0	−0.26	+11.8	0.23		
9%	−8%	+12%	−6%	−3%	−1%	−23%	−9%	−33%	+46%	+77%	+67%	−44%	−50%	−3%	−2%	−27%	+63%	−45%		
001	<0.001	<0.01	<0.4	>0.9	>0.9	<0.001	<0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.2	<0.01	<0.001	<0.001		
10.7	57.8	9.3	0.87	5.2	2,023	245	9.4	1.1	148	15.8	13.8	0.9	46	15.6	46	1.32	15.7	0.59		
12.7	55.0	8.8	0.69	5.6	1,659	213	8.0	1.0	75	9.5	6.6	1.6	47	14.2	43	0.56	9.4	0.84	37	480
16.1	53.6	11.3	0.70	2.2	3,537	686	3.0	0.6	157	17.7	17.7	0.6	8	17.1	52	0.55	52.3	0.17		
15.0	52.2	10.4	0.69	3.9	1,867	246	4.8	0.6	103	10.7	10.7	0.9	18	14.9	46	0.47	21.4	0.45	20	464

21. Cardiac respiratory quotient.
22. Cardiac output in liters per minute.
23. Total peripheral resistance in dynes per cm.$^{-5}$ per second.
24. Total pulmonary resistance in dynes per cm.$^{-5}$ per second.
25. Left ventricular work in kilogram meters per minute.
26. Right ventricular work in kilogram meters per minute.
27. Coronary blood flow in ml. per 100 gm. of myocardium per min.
28. Cardiac metabolic rate for oxygen in ml. per 100 gm. of myocardium per minute.
29. Cardiac metabolic rate for CO_2 in ml. per 100 gm. of myocardium per minute.
30. Coronary vascular resistance in units (MABP/CBF).
31. Stroke volume in ml.
32. Arterial hemoglobin in gm. per 100 ml. of blood.
33. Arterial hematocrit in per cent.
34. Coronary blood flow per beat (CBF ÷ rate).
35. Coronary blood flow per kilogram meter of left ventricular work.
36. Index of efficiency in kilogram meters of work done per ml. of $CMRO_2$.
37. Time from the injection of quinidine to the beginning of the second cardiac output.
38. Total dose of quinidine in mg.

TABLE II

Correlations between various factors *

	Control	Drug
CBF with CO	+0.6717§	+0.6198$^{\Delta}$
CBF with Rate	−0.1272	0.0000
CBF with CSO_2	+0.6532$^{\Delta}$	+0.4652
CBF with $CMRO_2$†	+0.4345	+0.6857§
CBF with CVR†	−0.9463‡	−0.7825‡
CBF with MABP	−0.3567	−0.0409
CBF with T.Pe.R.	−0.8129‡	−0.5925$^{\Delta}$
CBF with Card. R.Q.	−0.5344°	−0.5538°
$CMRO_2$ with LVW	+0.5344°	+0.5003
CVR with Rate	+0.0585	−0.1995
CVR with Card. R.Q.	+0.5411°	+0.3239
CVR with T.Pe.R.†	+0.8776‡	+0.6639§
CVR with CSO_2	−0.5998$^{\Delta}$	−0.4574
Ind. Eff. with $CMRO_2$†	−0.3854	−0.0292
Ind. Eff. with LVW†	+0.5563°	+0.8271‡
Ind. Eff. with St. Vol.	+0.2288	+0.8872‡
Ind. Eff. with Rate	+0.3064	−0.7822‡
Ind. Eff. with CO†	+0.5447°	+0.6085°
Ind. Eff. with CSO_2	+0.2799	+0.5902°

* Symbols are the same as in Table I.
Levels of significance are indicated as follows:

$p < 0.001$ ‡
$p < 0.01$ §
$p < 0.02$ $^{\Delta}$
$p < 0.05$ °

† Mathematical dependence of the two parameters accounts at least partly for the correlation since a common factor is used in their calculation.

The question of the role of the tachycardia *per se* in producing the increase in myocardial oxygen consumption arises. Laurent, Bolene-Williams, Williams, and Katz (14) observed an increase in myocardial oxygen consumption with an increase in heart rate at a relatively constant work load. The authors state that the increment of oxygen consumption by the myocardium was determined primarily by an increase in coronary flow rather than by any increase in myocardial arteriovenous oxygen difference. Preliminary studies in this laboratory utilizing the lightly anesthetized intact animal are in agreement with the observations of Laurent, Bolene-Williams, Williams, and Katz. Hence the tachycardia might contribute to the increase in coronary flow but apparently does not account for the increase in arterial-coronary sinus oxygen difference.

The dose of 15 mg. per kilogram of quinidine, demonstrated by Allen, Stutzman, Slocum, and Orth (9) to be effective in preventing cyclopropane epinephrine tachycardia in dogs, is roughly equivalent to a one-gram dose in a 70 kg. man, and is admittedly a large dose of quinidine. However, it does not differ greatly from the single oral dose of 1.2 Gm. given by Starr, Gamble, Margolies, Donal, Joseph, and Eagle (6) or the 0.8 Gm. single oral dose administered by Ferrer, Harvey, Werkö, Dresdale, Cournand, and Richards (7) in their study of patients. Undoubtedly, a higher concentration reached the cardiovascular system following intravenous administration as used in the present study than in the clinical experiments utilizing the oral route of administration. Gold and Modell (15) used increasing doses from 2 to 40 mg. per kg. intravenously in their study on dogs, representing a span on both sides of the dosage employed in this study.

Quinidine uniformly produces in dogs a tachycardia even at the 2 mg. per kg. dose level (15). Since tachycardia is not observed in human subjects there is undoubtedly a species difference with respect to this action of the drug. Only further studies in man could clarify the question of different effects in various species. However, similar hemodynamic studies performed in dogs with hexamethonium have been completely applicable to human subjects, except for the species difference of tachycardia in the former (11).

SUMMARY AND CONCLUSIONS

1. The hemodynamic and cardiac effects of administration of quinidine intravenously to 16 dogs have been studied. For technical reasons, 13 of these proved satisfactory.

2. Tachycardia and hypotension followed immediately after administration of the drug, and although the blood pressure returned to the control range before the second determination of cardiac output, the cardiac rate remained elevated.

3. Cardiac output determination twenty-five minutes after quinidine was not significantly different from the control measurement.

4. Although right ventricular work and total pulmonary resistance decreased, left ventricular work and systemic arterial resistance remained unchanged.

5. Coronary blood flow and cardiac metabolic rate for oxygen increased considerably whereas coronary vascular resistance and myocardial efficiency decreased markedly.

6. The increase in myocardial oxygen consumption was associated with an increase in coronary

blood flow and an increase in arterial-coronary sinus oxygen difference.

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EARLY EFFECTS OF FAT INGESTION ON LIPIDS AND LIPOPROTEINS OF SERUM IN MAN

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The mechanisms of fat transport in the blood are still poorly understood. Recent studies have shown that all lipids in blood plasma exist as lipoprotein complexes, that a broad spectrum of such complexes exists, and that groups of these complexes can be separated from each other by various physical means. Application of these separation methods to the study of chemical changes within lipoprotein classes should provide greater understanding of fat transport.

The chylomicronemia produced by ingestion of fat in man is accompanied chiefly by an increased concentration of triglycerides, the chief components of chylomicra (1, 2) in the serum. Phospholipid concentration also increases significantly (3), but free and ester cholesterol concentrations increase slightly (4) or not at all (5, 6). No changes have been reported in the concentrations in serum of high density and S_f 0-30 classes of lipoproteins after ingestion of fat, but the concentration of the higher S_f classes increases. There appears to be a progressive shift with time in the concentrations of these very low density lipoprotein classes in the direction of greater density (7). Chylomicra and lipoproteins of high S_f rates consist chiefly of triglycerides with lesser quantities of phospholipids, cholesterol esters, unesterified cholesterol, and protein (8). Thus, the serum lipid changes found after ingestion of fat might be explained exclusively by increased concentrations of chylomicra and lipoproteins having S_f rates exceeding 30.

To define further the effects of ingestion of fats on serum lipids and lipoproteins, alterations in various lipid constituents of ultracentrifugally separated lipoprotein classes of serum were measured in healthy young adults following the ingestion of high fat meals. These studies have shown that the concentrations of major chemical constituents of both high and very low density lipoproteins in serum are increased after ingestion of fat.

METHODS

Experimental subjects. Healthy young adults, who had undergone thorough physical and laboratory examinations and who had no apparent systemic disease, were hospitalized in a metabolic ward. Other subjects were professional and technical personnel who were not examined but who had no known systemic disease.

Diets. Hospitalized subjects were given measured diets containing varying quantities of fat. After a 12 to 15-hour fast, they were given high fat breakfasts containing 1.5 gram of fat per kgm. of body weight as calculated from standard tables. The composition of these meals is listed in Table I. Occasional transient nausea was the only apparent adverse effect of eating this quantity of fat. The subjects were allowed water but no food during the next 8 hours. Between the eighth and tenth hour they were given a supper supplying 10 calories per kgm. of body weight which contained practically no fat. Only water was permitted thereafter until completion of the test.

Blood samples and analytical procedures. Blood samples were taken from an antecubital vein and allowed to clot. The serum was extracted with ethanol-acetone (1:1 v/v) or chloroform-methanol (2:1 v/v). Two ml. of serum contained in a calibrated hypodermic syringe was forcefully ejected into about 45 ml. of solvent through a 23-gauge needle, and additional solvent added to give a final volume of 50 ml. Analyses for cholesterol (9), lipid phosphorus (10), and total lipid (11) were done directly on the ethanol-acetone extract after the precipitated proteins had been centrifuged down. The chloroform-methanol extract was treated further by a modification of the procedure described by Sperry and Brand (12). The extract was separated into two phases by the addition of 10 ml. of distilled water. The two phases were mixed by inversion and allowed to separate overnight. The upper phase contains the crystalloids of serum, and the lower phase the lipids. The final volume of the lower phase was 36 ml.

Direct comparison of results obtained by the two extraction procedures demonstrated that the yields of total and free cholesterol and of lipid phosphorus were identical,

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TABLE I

COMPOSITION OF MEALS USED TO PRODUCE ALIMENTARY LIPEMIA

Meal No.	Composition per Kgm. Body Weight		Preparation
1	36% cream	4.0 ml.	
	skimmed milk	4.0 ml.	
	cocoa	0.12 gm.	homogenized
	sucrose	0.25 gm.	
2*	whole egg	4.5 gm.	egg beaten in
	oleomargarine	0.8 gm.	melted oleo-
	bacon, limp fried	0.5 gm.	margine and fried
3	corn oil	1.5 gm.	
	skimmed milk powder	0.5 gm.	
	sucrose	0.15 gm.	homogenized
	water	3.0 ml.	
	vanilla (to flavor)		

* Contains only about 0.05 gm. carbohydrate

whereas the calculated triglyceride content (11) was about 100 mg. per 100 ml. of serum higher in the ethanol-acetone extract. This results from the fact that certain crystalloids, principally chloride ion, contaminate the ethanol-acetone extract and contribute to the apparent total lipid concentration. Consequently, 100 mg. has been subtracted from all triglyceride values obtained by this method. Crystalloids are practically absent from the chloroform-methanol extract. Unesterified fatty acids are distributed between the two phases of this extract (13), but virtually no other lipids are present in the polar phase. "Triglyceride" values given for the chloroform-methanol extract therefore represent glyceryl esters of fatty acids (chiefly triglycerides [14]) plus a portion of the unesterified fatty acids.

Separation and analysis of lipoprotein fractions. Lipoprotein fractions were separated from serum as described previously (15). The densities and nature of the fractions are given in Table II. Aliquots of Fraction I + II were extracted in chloroform-methanol. The other fractions were extracted in ethanol-acetone. Since Fraction I + II contains about 50 per cent triglycerides and practically no unesterified fatty acids (13), triglyceride values for this fraction are considered to be more accurate than those obtained on whole serum. Analyses were done as on whole serum.

TABLE II

ULTRACENTRIFUGALLY SEPARATED LIPOPROTEIN FRACTIONS

Fraction	Nomenclature	Density	Electrophoretic Mobility (starch)
I + II	Very low density lipoproteins, including chylomicrons (Sf >10)	<1.019	Alpha-2 globulin
III	Low density lipoproteins (Sf 0-10)	1.019-1.063	Beta-1 globulin
IV	High density lipoproteins	1.063-1.21	Alpha-1 globulin
V	Unesterified fatty acids	>1.21	Albumin
	+		+
	Residual serum proteins		All components

RESULTS

Serum lipids

The initial studies were carried out on subjects in a metabolic ward to determine the effects of previous diet on the nature and duration of hyper-

lipemia following ingestion of fat. Four subjects were given, in succession, isocaloric diets which contained 1.0, 0.1 and 2.0 grams of fat per kgm. body weight. The first diet was maintained for 2 weeks, and the second and third for 1 week each. Fat breakfast No. 1 was given at the end of each week of study. The results, tabulated in Table III, show that the magnitude of the increment in serum triglyceride concentration was not related consistently to the nature of the diet during the week preceding the test; however, the hyperlipemia frequently persisted longer when the subjects were given the high and low fat diets than when they received the moderate fat diet. Of further interest is the fact that the serum triglyceride concentration was usually lower 12 hours after the fat meal than just before or 24 hours after its ingestion, regardless of the fat content of the diet. For all diets the mean triglyceride concentration at 12 and 24 hours, respectively, was 56 and 79 mg. per 100 ml. of serum, with a probability of less than 0.01 that the difference observed was the result of

TABLE III

ALTERATIONS IN WHOLE SERUM LIPID CONCENTRATIONS FOLLOWING INGESTION OF 1.5 Gm. CREAM FAT PER Kg. BODY WEIGHT

Patient	Test No.*	Total Cholesterol				Phospholipids				Glycerides			
		1	2	3	4	1	2	3	4	1	2	3	4
	Hours												
G.W. Male 20	0	134	122	93	135	166	175	143	175	60	75	95	45
	4	128	129	94	135	177	190	163	199	225	200	175	290
	8	132	130	93	145	197	212	159	228	150	190	110	210
	12	134	120	103	132	179	190	156	180	20	60	45	60
	24	129	121	98	136	173	170	143	169	55	90	40	65
G.C. Male 20	0	193	154	121	164	205	183	159	195	80	75	100	50
	4	182	160	125	182	231	218	191	226	290	200	190	190
	8	198	153	126	170	229	214	195	231	55	100	145	140
	12	181	159	121	174	199	210	172	211	40	60	75	45
	24	182	140	114	182	199	180	159	211	90	90	110	95
F.B. Female 24	0	151	153	111	135	185	179	162	175	75	50	120	85
	4	170	167	128	145	229	216	200	210	145	120	235	280
	8	167	170	132	150	218	230	218	217	90	85	245	170
	12	157	169	111	158	198	205	175	226	65	50	40	100
	24	166	---	109	140	190	---	155	170	75	--	85	80
P.M. Female 19	0	174	175	129	157	189	185	180	188	65	45	75	100
	4	189	175	147	178	225	212	209	235	105	90	155	205
	8	178	181	155	178	218	225	228	230	70	65	155	70
	12	183	175	138	166	205	200	202	206	50	20	70	55
	24	175	---	134	158	200	---	166	182	70	--	85	75

* Test No. 1: On isocaloric diet containing 1 gm. fat per kg. body weight for 1 week

Test No. 2: On isocaloric diet containing 1 gm. fat per kg. body weight for 2 weeks

Test No. 3: On isocaloric diet containing 0.1 gm. fat per kg. body weight for 1 week

Test No. 4: On isocaloric diet containing 2 gm. fat per kg. body weight for 1 week

chance. The concentration of phospholipids rose uniformly and significantly during fat absorption. The increment in phospholipid concentration was not related consistently to the increment in concentration of triglycerides, and phospholipid concentration usually remained elevated after triglyceride concentration had returned to baseline, or lower, values. Twelve hours after the ingestion of fat, triglyceride concentration was below the baseline value 13 times in 16 tests, whereas phospholipid concentration was more than 12 mg. per 100 ml. of serum higher than the baseline value 14 times in 16. The concentration of total cholesterol in the serum usually showed no consistent increase in the two male subjects. In the females cholesterol concentration rose at least 15 mg. per 100 ml. of serum following 7 of the 8 fat meals.

Lipoprotein fractions

Five additional subjects who were not on measured diets were studied after the ingestion of a single high fat meal. Meals No. 1 and 2 were

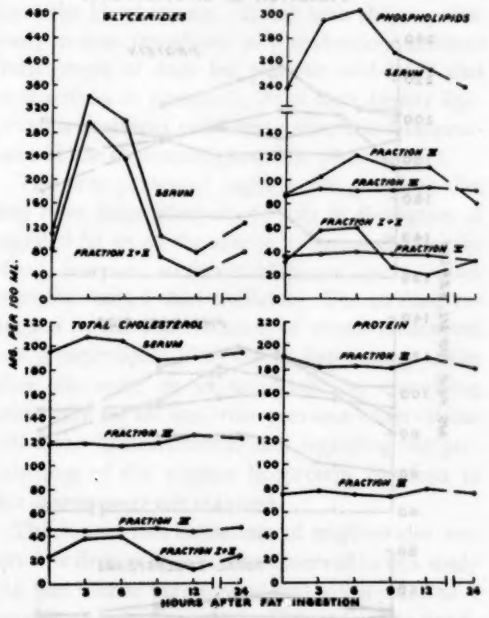

FIG. 1. ALTERATIONS IN THE CONCENTRATIONS OF SERUM LIPIDS AND CHEMICAL CONSTITUENTS OF SERUM LIPOPROTEIN FRACTIONS FOLLOWING INGESTION OF 1.5 GRAM OF FAT PER KGM. BODY WEIGHT IN A HEALTHY ADULT

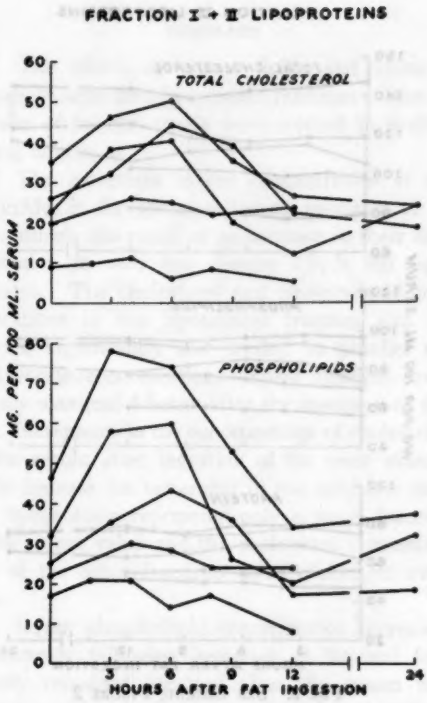

FIG. 2. ALTERATIONS IN THE CONCENTRATIONS OF CHEMICAL CONSTITUENTS OF SERUM LIPOPROTEIN FRACTIONS FOLLOWING INGESTION OF 1.5 GRAM OF FAT PER KGM. BODY WEIGHT IN FIVE HEALTHY ADULTS

used twice, and meal No. 3 once. The design of the experiments was the same as in the 4 subjects studied previously. Lipoprotein fractions were separated from aliquots of each serum sample and analyzed for lipid and protein constituents. Similar alterations in the various fractions were observed with the three types of fat-rich meal. Detailed results of one study are shown in Figure 1. The significant features are: 1) Alterations in serum triglyceride concentration reflected solely changes in triglycerides of Fraction I + II; 2) changes in serum total cholesterol concentration were relatively slight, but the small quantity of cholesterol in Fraction I + II was almost doubled; 3) changes in serum phospholipid concentration reflected the sum of changes in Fractions I + II and IV, and the elevation of Fraction IV persisted longer than that of Fraction I + II; 4) changes in protein concentration in Fractions III and IV were slight; 5) changes in the three major lipid

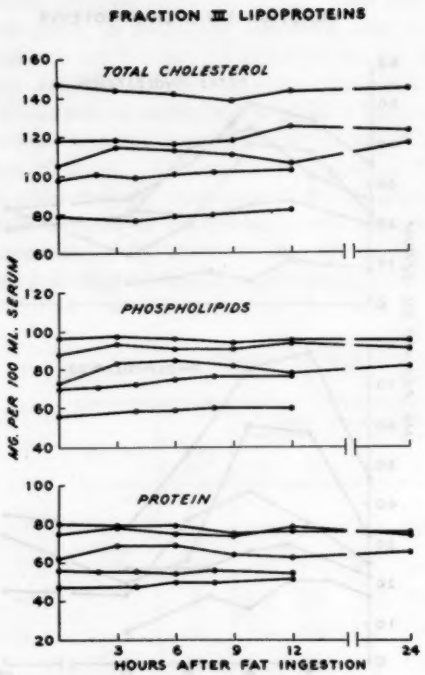

FIG. 3. SEE LEGEND, FIGURE 2

components of Fraction I + II tended to parallel each other, but the fall in the concentration of triglycerides preceded that of cholesterol and phospholipids; 6) the concentrations of Fraction I + II components were lower after 12 hours than after 24 hours. Alterations in measured components of the lipoproteins of the five subjects are shown in Figures 2 to 4. Although not shown in the figures, the magnitude of the increments in serum and Fraction I + II triglyceride concentrations tended to parallel that of the other lipid constituents of this fraction. Alterations in the concentrations of components of Fraction III were slight. In Fraction IV, phospholipid concentration rose consistently and tended to remain elevated 12 hours after the ingestion of fat, whereas total cholesterol and protein concentrations rose in only 3 of the 5 tests. The ratio of cholesterol to phospholipid in this fraction fell during fat absorption.

DISCUSSION

The demonstration that the increment in serum triglyceride concentration after ingestion of fat is contained entirely in chylomicra and/or very low density lipoproteins is in accord with current concepts of exogenous fat transport. It has been shown previously in dogs by Swank and Wilmot (16) and in man by van Eck, Peters, and Man (17) that the bulk of the increment in serum triglyceride concentration after the ingestion of fat can be layered on the surface of the serum by relatively gentle centrifugation. In their studies no layering of cholesterol or phospholipids was demonstrated, although later studies have shown that equivalent centrifugation layers some of the cholesterol and phospholipids of human chyle (18), a result to be expected from the known composition of chylomicra (8). The present results neither support nor contradict the concept that chylomicra are degraded in blood plasma with the production of progressively higher density lipoproteins (7), but the fact that the concentration of cholesterol and of phospholipids in Fraction I + II sometimes fell later than that of the triglycerides is consistent with such an interpretation.

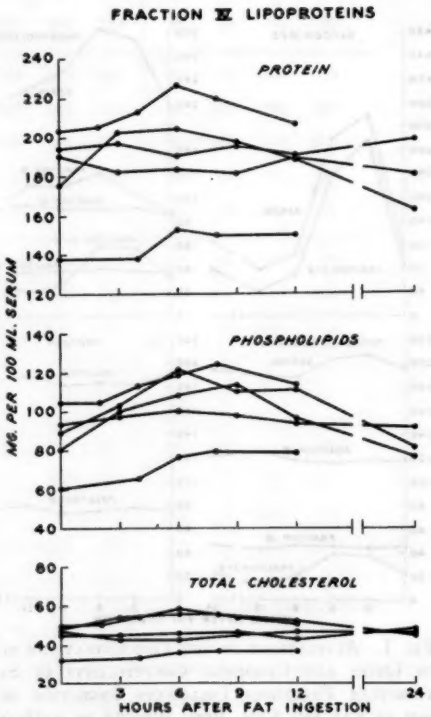

FIG. 4. SEE LEGEND, FIGURE 2

The delayed fall in the concentration of serum phospholipids following ingestion of fat is clearly the result of increased phospholipid concentration in high density lipoproteins (*cf.* Figure 1). This finding is not in accord with current concepts of lipoprotein alterations during transport of exogenous fat in the blood, although Swank and Wilmot found that the increased concentration of phospholipids in the serum of dogs absorbing fat remained in the infranatant serum after flotation of chylomicra (16).

The mechanism of the rise in phospholipid concentration in high density lipoproteins is unknown. Net transfer of phospholipid from Fraction I + II appears unlikely since there was no similar increase in phospholipid of Fraction III lipoproteins. That the increment resulted from an augmented rate of transfer of high density lipoproteins from thoracic duct lymph also appears unlikely because the other constituents of this fraction frequently showed no increase. It seems most probable that the changes observed are related to the further metabolism of exogenous fatty acids after they first leave the blood stream. It has been shown previously in dogs transfused with chylomicra obtained from lymph of dogs fed palmitic acid-1-C^{14} that radioactivity in phospholipids of high density lipoproteins continues to increase after the disappearance of the chylomicra from the plasma (19).

The data presented suggest that previous diet may have some effect on the rate of absorption of ingested fat or on the rate of its removal from the blood; however, no firm conclusions can be drawn from the limited data available. The striking reduction in the concentration of serum cholesterol and phospholipids observed in four young adults after one week on an isocaloric diet containing practically no fat confirms previous observations (20–22). Unfortunately, data regarding the participation of the various lipoprotein fractions in this change were not obtained.

The lowest concentrations of triglycerides and very low density lipoproteins observed in this study (12 hours after ingestion of fat) occurred 3 to 4 hours after a fat-free meal. This raises the possibility that the availability of energy from sources other than fat may influence the concentration of these lipoproteins. This problem is the subject of the succeeding paper.

SUMMARY

1. The effects on serum lipids and ultracentrifugally separated lipoprotein fractions of the ingestion of fat-rich meals were studied in healthy young adults.

2. The increment in the concentration of triglycerides in the serum following ingestion of fat was entirely the result of an increase in their concentration in very low density ($S_f > 10$) lipoproteins. The cholesterol and phospholipid concentrations in this lipoprotein fraction also increased significantly and tended to parallel the triglyceride concentration. These changes were usually maximal 4 hours after the ingestion of fat.

3. Increments in the concentration of cholesterol in the serum after ingestion of fat were usually slight because the increment in the very low density lipoproteins represents only a small fraction of the serum value and the cholesterol concentration of the other fractions usually changes very little.

4. Serum phospholipid concentration increased significantly following ingestion of fat and frequently remained elevated after the serum triglyceride concentration had returned to baseline, or even lower, levels. The increase reflected changes in both the very low density and high density lipoprotein fractions. The delayed fall was the result of the persistence of the increased concentration of high density lipoprotein phospholipids after the visible lipemia had disappeared. Lesser increases in the concentration of high density lipoprotein cholesterol and protein may also occur after ingestion of fat.

5. These findings are discussed in relation to current concepts of exogenous fat transport.

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EARLY EFFECTS OF FASTING AND OF CARBOHYDRATE INGESTION ON LIPIDS AND LIPOPROTEINS OF SERUM IN MAN

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(Submitted for publication January 21, 1957; accepted February 28, 1957)

It is generally assumed that plasma lipoproteins function as carriers in the transport of fatty acids to and from the liver, fat depots, and peripheral tissues, but the roles of the various lipoprotein classes in this regard are not established. A previous study showed that the concentration of triglycerides in serum was lower several hours after the ingestion of a meal containing no fat than after an overnight fast (1). In the present study large quantities of glucose were fed to fasting young adults; the resulting alterations in various lipid constituents of ultracentrifugally separated lipoprotein classes were compared with changes observed during comparable periods of continued fasting. These comparisons showed that the concentrations of all major lipid constituents of the very low density lipoproteins of serum fall significantly after the ingestion of glucose. This finding suggests that very low density lipoproteins have a significant role in the transport of fatty acids derived from endogenous sources.

METHODS

Experimental subjects and procedure

Seven adults who had no known systemic disease were studied during 2 periods, each preceded by a 12 to 15-hour fast (water allowed *ad libitum*).

Period I. The subjects continued to fast for an additional 11 hours, during which serial blood samples were withdrawn.

Period II. The same subjects fasted for 3 additional hours, after which they were fed 100 grams of glucose as lemonade at hourly intervals for 4 hours. Two or three hours thereafter they were allowed to eat a fat-free supper. Serial blood samples were withdrawn at intervals during the test and again the next morning before breakfast (*cf.* Figure 1). In three of the subjects period I preceded period II. In the other four subjects the periods were reversed.

Analytical methods

These have been described in the preceding paper (1). All serum samples were extracted in chloroform-methanol, 2:1, v/v. Serum total protein concentrations were determined on all samples by the biuret method (2), and lipoprotein lipid and protein concentrations were corrected to a constant total protein concentration to minimize changes resulting from fluctuations in the concentration of plasma proteins.

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RESULTS

Changes in total lipid concentration in Fraction I + II lipoproteins ($S_f > 10$) in the seven subjects are shown in Figure 1. Mean values are given in Table I. As shown in Table I, the concentration of total lipids fell slightly during the first 3 hours of the test. With continued fasting, the changes were variable, but resulted in no significant change in mean values. When glucose was administered, providing 1600 calories over a period of 4 hours, total lipid concentration fell consistently, irrespective of the initial concentration, and was lowest 8 hours after glucose was first administered. The following morning, after another 12 to 15 hours of fasting, the levels had risen consistently in the five subjects tested.

In four of the subjects (Table II) detailed lipoprotein analyses were carried out during both periods. In the fasting state there were no consistent changes in any fraction, except for a slight increase in Fraction IV (high density lipoprotein) phospholipids. After ingestion of glucose, all lipid constituents in Fraction I + II were reduced. In general, the decrease was most marked in triglycerides, slightly less in phospholipids, and least in total cholesterol. In Fraction III (S_f 0-10 lipoproteins) there was a slight fall in the mean values for all constituents. In every instance Fraction IV phospholipid concentration fell 5 and 8 hours after ingestion of glucose; no comparable changes occurred in cholesterol or protein concentration.

DISCUSSION

Rubin and Aladjem (3), using the analytical centrifuge, studied lipoprotein concentrations in six healthy subjects during 96 hours of fasting.

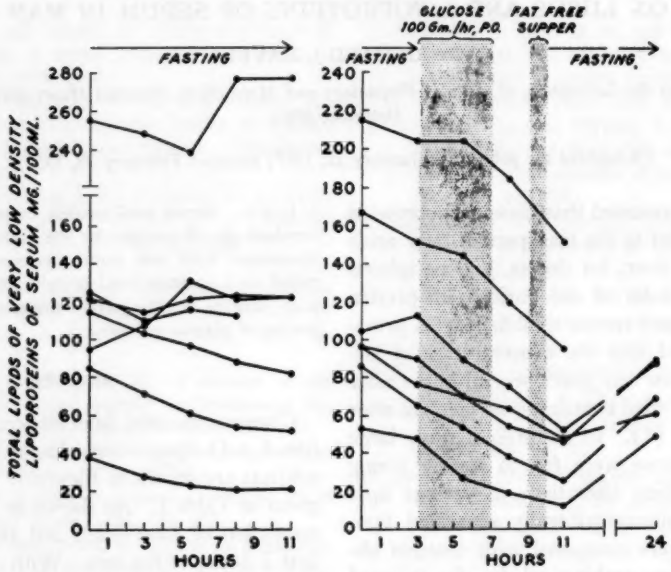

FIG. 1. ALTERATIONS IN VERY LOW DENSITY (FRACTION I + II) LIPOPROTEIN TOTAL LIPID CONCENTRATIONS IN HEALTHY ADULTS DURING FASTING AND AFTER INGESTION OF GLUCOSE

All tests begun after preliminary 12 to 15-hour fast.

In accord with the present data, their results showed that the concentration of S_f 12-400 (Fraction I + II) lipoproteins was variable during the first 24 hours of fasting and that the concentrations of S_f 0-12 (Fraction III) and high density (Fraction IV) lipoproteins did not change significantly. After 96 hours the concentrations of S_f 12-400 and 0-12 lipoproteins had increased significantly,

TABLE I

MEAN VALUES FOR TOTAL LIPID IN FRACTION I + II LIPOPROTEINS AFTER FASTING AND GLUCOSE INGESTION IN SEVEN HEALTHY ADULTS

Hour of Test	0	3	5-1/2	8	11
Fasting:					
Mean	119	112	113	114	113
Difference from 3-hour value			+1	+2	+1
Standard error*			5	6	8
P			0.9 > P > 0.8	0.8 > P > 0.7	0.8 > P > 0.7
Glucose:					
Mean	117	107	93	79	61
Difference from 3-hour value			-14	-28	-46
Standard error*			4	2	4
P			0.02 > P > 0.01	< 0.01	< 0.01

* $\left(\frac{\Sigma(d-\bar{d})^2}{n(n-1)}\right)^{1/2}$

TABLE II

CONCENTRATIONS OF SERUM LIPOPROTEIN CONSTITUENTS AFTER FASTING AND GLUCOSE INGESTION IN HEALTHY ADULTS

Hour of Test		Fasting 0	3	5-1/2	8	11	Glucose 0	3	5-1/2	8	11	24
Fraction I + II												
Total Cholesterol	RH	16	18	20	21	22	17	15	12	11	8	11
	LN	27	23	28	25	--	23	22	21	18	13	19
	CD	66	66	61	69	73	52	50	50	46	45	--
	MG	18	16	14	14	12	17	14	13	10	8	16
	Mean:	32	31	31	32	--	27	25	24	21	19	--
Phospholipids	RH	22	25	29	25	30	21	20	16	13	10	13
	LN	30	28	32	28	--	26	24	22	18	13	22
	CD	54	53	56	62	62	49	46	44	38	32	--
	MG	26	23	21	20	17	23	19	16	13	9	22
	Mean:	33	32	35	34	--	30	27	25	21	16	--
Glycerides	RH	53	58	69	62	57	52	48	37	34	26	31
	LN	54	51	61	49	--	46	44	34	22	17	37
	CD	89	89	101	126	117	97	91	88	71	45	--
	MG	64	57	51	46	41	50	43	33	26	22	50
	Mean:	65	64	71	71	--	61	57	48	38	28	--
Fraction III												
Total Cholesterol	RH	109	111	111	106	109	135	133	133	131	129	128
	LN	90	92	97	100	---	98	93	92	95	93	94
	CD	117	112	108	108	109	116	117	117	115	112	--
	MG	86	87	95	96	89	92	90	94	89	88	91
	Mean:	100	100	103	100	--	110	108	109	108	106	--
Phospholipids	RH	75	80	78	74	76	92	92	92	87	90	93
	LN	68	71	75	76	--	72	66	67	66	66	70
	CD	84	81	78	82	79	80	79	77	76	76	--
	MG	64	64	66	69	68	64	63	64	62	60	63
	Mean:	73	74	74	75	--	77	75	75	73	73	--
Protein	RH	63	63	63	57	60	75	73	73	71	69	72
	LN	58	58	60	60	--	52	54	48	52	51	53
	CD	78	75	68	72	69	73	71	69	70	71	--
	MG	54	53	56	55	57	53	53	53	52	51	54
	Mean:	63	62	62	61	--	63	63	61	61	61	--
Fraction IV												
Total Cholesterol	RH	41	38	37	39	39	41	38	39	41	40	37
	LN	36	36	37	37	--	40	40	40	43	41	43
	CD	42	41	40	38	38	40	37	36	36	37	--
	MG	44	44	45	44	45	48	47	47	47	45	46
	Mean:	41	40	40	40	--	42	41	41	42	41	42
Phospholipids	RH	71	70	71	76	79	76	70	68	67	65	67
	LN	66	69	75	76	--	75	75	74	72	70	81
	CD	82	80	81	81	81	74	72	67	67	63	--
	MG	81	83	85	86	86	80	79	79	74	71	84
	Mean:	75	76	78	80	82	76	74	72	70	67	77
Protein	RH	156	151	158	162	170	176	---	---	176	172	172
	LN	174	171	172	170	---	164	169	164	163	170	177
	CD	164	163	164	164	168	154	153	151	150	151	---
	MG	185	203	203	201	191	188	184	190	188	181	197
	Mean:	170	172	174	174	---	173	---	---	168	169	---

whereas the concentration of the high density lipoproteins had remained unchanged. They then administered 1 gram of sucrose per kgm. of body weight; no significant changes occurred in the next 3 hours, but normal values were found 24 hours later after the subjects had resumed their usual diet. The failure of Rubin and Aladjem to observe a prompt decrease in the concentration of S_f 12-400 lipoproteins after ingestion of sucrose may have resulted from the relatively small quan-

tities of carbohydrate they administered. In the present study more than enough glucose was supplied over a period of 4 hours to satisfy the subjects' caloric requirements, and it is likely that they were utilizing carbohydrate as the major source of energy for at least 6 hours. The next morning they undoubtedly were deriving considerable energy from fat combustion, and the concentration of Fraction I + II lipoproteins rose significantly, although no fat had been eaten in the interim.

The results presented demonstrate the considerable lability of very low density lipoproteins independent of chylomicron transport, and suggest that their concentration, like that of ketone bodies and unesterified fatty acids (4, 5), may depend on the availability of carbohydrate for energy production. The marked increase in the concentration of this lipoprotein fraction in diabetic acidosis (6) and its rapid fall with treatment, along with the decrease in ketone bodies and unesterified fatty acids (7), support this concept.

Others have noted the lability of the very low density lipoproteins. Chandler, Lawry, Potee, and Mann (8) found that the diurnal variability in the concentration of S_f 20-100 but not of S_f 12-20 lipoproteins exceeded any variation that could be attributed to technical error, but observed no diurnal trends. This is not surprising, for the opposing effects of fat and carbohydrate ingestion would tend to be neutralized after a mixed meal. On the other hand, Boyd (9), in a study of subjects on mixed diets supplying 2000 to 2500 calories per day, observed a diurnal variation in the concentration of neutral fat which reached a nadir at 5 P.M. In this case, the effect of carbohydrate on very low density lipoproteins probably outweighed the effect of fat. Critical data concerning the importance of very low density lipoproteins in the transport of fatty acids derived from endogenous sources remain to be obtained. Available data regarding fatty acid transport pertain only to whole serum. Harper, Neal, and Hlavacek (10) observed in dogs that the turnover of the nonphospholipid fatty acid fraction of plasma (triglycerides, cholesterol esters, and unesterified fatty acids) was considerably more rapid than that of the phospholipid fatty acid fraction. These observations have been confirmed in man by Lipsky, McGuire, Bondy, and Man (11). Recent studies indicate that unesterified fatty acids may account for a significant portion of fatty acid transport during fasting (5) and during removal of chylomicra from the plasma (12). In chylomicra, triglycerides are the chief vehicle for fatty acid transport (13), but their role in the transport of endogenous fatty acids remains to be clarified. Since the lipid of Fraction I + II lipoproteins consists mainly of triglycerides, it appears likely that the lability of this fraction is related to their turnover.

The prolonged effects of fasting and of ingesting fat-free foods must be differentiated from those occurring during the first 24 hours. Kartin, Man, Winkler, and Peters (14), in a study of the effects of prolonged fasting on serum lipid concentrations in human subjects, found that free and esterified cholesterol and phospholipid concentrations rose consistently and that neutral fats showed variable changes. In their series the effects of fasting were complicated by concomitant water deprivation, and the extent of hemoconcentration in their subjects is not known. In contrast to Rubin and Aladjem's report that very low density lipoproteins were increased after 72 hours of fasting (3), this study did not imply an elevation of this fraction during more prolonged fasting. The changes in serum lipid concentrations (as well as ketosis) observed by Kartin and his colleagues could be prevented by the ingestion of 100 grams of carbohydrate daily.

Prolonged ingestion of fat-free diets results in rapid fall of cholesterol and phospholipid concentrations within one week (15–17), even in the absence of weight loss, but may not appreciably change fasting serum triglyceride concentrations. It is apparent that lipoproteins other than those of very low density must be involved in these changes. On the other hand, when an excess of calories in the form of carbohydrates and proteins is supplied over a period of several days, S_f 12-400 lipoprotein concentrations rise strikingly (18). This suggests that transport of newly synthesized fatty acids to depots may take place via very low density lipoproteins.

The physiologic roles of Fraction III and IV lipoproteins are not clarified by the present study. A close relation between Fraction I + II and III is suggested by the fact that a) their protein moieties have the same N terminal amino acid residue (19), and b) the concentrations of these fractions tend to vary inversely in certain pathologic states, such as idiopathic hyperlipemia (20) and the nephrotic syndrome (21). The protein moiety of

Fraction IV has a different N terminal amino acid residue (19) and therefore cannot be a precursor or product of the other fractions. The fall in phospholipid concentration in Fraction IV after ingestion of glucose and the slight rise observed during fasting suggest a possible role for this fraction in fatty acid transport as well.

SUMMARY

1. The concentrations of lipid and protein constituents of ultracentrifugally separated serum lipoprotein fractions were studied in seven healthy adults during short periods of fasting and after ingestion of a large quantity of glucose. To minimize the effects of chylomicronemia, a 12 to 15-hour interval of fasting preceded each period.

2. The total lipid concentration in very low density ($S_f > 10$) lipoproteins was variable during fasting, but fell consistently after ingestion of 400 grams of glucose over a 4-hour period. The levels 5 and 8 hours after starting glucose feeding were significantly lower than those after comparable periods of fasting. These changes resulted from reductions in all lipid constituents of this lipoprotein fraction, but the mean per cent fall was greatest in triglycerides, less in phospholipids, and least in cholesterol.

3. No significant changes occurred in the concentrations of lipid constituents or protein in the major low density (S_f 0-10) lipoproteins during either of the periods of study.

4. The concentrations of cholesterol and protein in high density lipoproteins did not change during the periods of study, but the concentration of phospholipids rose slightly during continued fasting and fell during the period following ingestion of glucose.

5. The possible implications of these findings in relation to lipid transport in the blood are discussed.

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THE HEMODYNAMIC EFFECTS OF HEXAMETHONIUM BROMIDE IN THE DOG, WITH SPECIAL REFERENCE TO "SPLANCHNIC POOLING" [1]

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Arterial hypotension develops during the action of hexamethonium in man and the dog because cardiac output decreases in association with a fall in peripheral and central venous pressures (1–5). The drug does induce vasodilation in the skin, muscle, and brain; but vasoconstriction occurs in other areas so that the net total peripheral vascular resistance remains unchanged or actually rises. Since there is little evidence of myocardial dysfunction, diminished venous return of blood to the heart must be implicated as the cause for the fall in output. This response may be attributed 1) to a slight but widespread change in venous tone that results in a generalized retardation in venous flow, 2) to local "pooling" of blood in venous reservoirs or 3) to both. The increased distensibility of the digital veins in human subjects following intravenous administration of hexamethonium (6) gives some support to the first possibility, but the digital vasculature is highly specialized and may not faithfully reflect changes elsewhere. Moreover, a larger quantity of blood is held in other parts of the venous system where even limited changes in tone might be expected to have a correspondingly greater effect upon venous pressure and return. The splanchnic venous bed is particularly important in this respect because it accommodates as much as 20 per cent of the total blood volume at rest in both man and dog. Although hexamethonium does not seem to affect splanchnic vascular tone since splanchnic blood flow falls in proportion to the fall in arterial pressure (1, 7), it is possible that the splanchnic veins are influenced independently of arterioles so that venous distensibility and capacity are altered in the absence of a change in arteriolar resistance. To test this possibility, splanchnic blood flow and splanchnic blood volume were measured by indirect methods in dogs during the fall in cardiac output and arterial pressure produced by hexamethonium.

METHODS

Thirteen mongrel dogs (10.2 to 17.7 Kg.), five of which had been splenectomized at least two weeks previously, were studied under Nembutal® anesthesia (30 mg. per Kg.). Catheters were inserted by way of an internal jugular vein into a left hepatic vein and the right heart and left in place throughout the study. Both femoral arteries were cannulated, one for pressure measurement and the other for blood sampling. After control determinations of mean arterial blood pressure, cardiac output, splanchnic blood flow, total blood volume and circulating splanchnic blood volume were obtained, a single dose of hexamethonium bromide (0.6 to 0.8 mg. hexamethonium ion per Kg.) was given intravenously. All measurements, with the exception of total blood volume, were repeated following stabilization of the arterial pressure at a hypotensive level (60 to 95 millimeters of mercury). The cardiac output was determined approximately 15 to 20 minutes, and splanchnic blood flow and splanchnic blood volume 20 to 30 minutes after administration of the drug.

Mean arterial blood pressure was either measured directly with a mercury manometer or determined by planimetric integration of pulse wave tracings recorded photographically from a Statham pressure transducer. Cardiac output was measured by the direct Fick method on the basis of values for oxygen consumption (Benedict-Roth spirometer) and oxygen contents [Van Slyke and Neill (8)] of femoral arterial and right ventricular blood sampled simultaneously. Splanchnic blood flow (ESBF) was estimated by Bromsulfalein (BSP) extraction and clearance (9). Plasma volume was measured by dilution of I^{131}-labelled human serum albumin (IHSA) and total blood volume calculated using the arterial hematocrit.

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TABLE I

Effect of hexamethonium on arterial pressure, cardiac output, total peripheral resistance, estimated splanchnic blood flow, splanchnic vascular resistance and circulating splanchnic blood volume in intact and splenectomized dogs *

Dog No.	Wt. Kg.	C_6 dose mg. ion	Hct %	MAP mm. Hg	O_2 consumption ml./min.	A−MV O_2 diff. vol. %	CO L./min.	TPR A.U.	ESBF ml./10 Kg./min.	SVR A.U.	SMCT sec.	SBV ml./10 Kg./min.	TBV ml./10 Kg.	SBV/TBV ×100 %
						A. Intact								
1	13.2	Control	39.5	140	126				373	17.8	30.2	188	1,233	15.2
		7.5	36.5	78	71				276	12.8	60.0	276		22.4
2	16.4	Control	36.5	122	89	5.6	1.58	4.6	291	15.3	26.4	128	864	14.9
		10.0	32.5	75	81	8.6	.94	4.8	269	10.2	38.5	173		20.0
3	15.5	Control	37.0	117	92				473	9.6	52.8	416	1,450	28.7
		10.0	37.0	86	99				373	8.9	67.1	418		28.8
4	11.8	Control	46.5	137	68	2.3	2.93	2.8	519	13.4	14.6	126	1,362	9.3
		7.5	43.5	87	89	3.6	2.48	2.1	608	7.3	21.9	222		16.3
5	15.9	Control	37.5	113	101	3.8	2.65	2.6	470	9.1	31.6	247	1,654	15.0
		10.0	37.5	92	83	4.2	1.96	2.8	362	9.6	56.9	344		20.8
6	14.1	Control	55.0	114	152	11.1†	1.37	5.0	383	12.7	33.2	212	1,167	18.2
		10.0	55.0	76	100	10.5†	.96	4.8	312	10.4	82.0	427		36.6
7	15.6	Control	44.5	119	80	6.3	1.29	5.5	209	21.9	42.3	147	1,665	8.9
		10.0	44.5	68	65	5.4	1.19	3.4	204	12.8	68.2	232		13.9
8	13.6	Control	39.5	129	62	4.0	1.54	5.0	685	8.3	18.4	209	970	21.5
		10.0	44.0	68	43	5.3	.81	5.0	386	7.8	51.4	331		34.1
						B. Splenectomized								
9	10.7	Control	51.5	83	67	3.9	1.72	2.9	540	8.6	20.0	179	1,187	15.1
		12.5	42.5	10	58	7.2	.72	0.8						
				61					232	14.7	76.0	294		24.7
10	12.5	Control	51.0	137	68	2.9	2.34	3.5	372	17.7	32.8	203	1,160	17.5
		10.0	50.5	95	62	3.9	1.58	3.6	290	15.7	62.4	304		26.2
11	11.3	Control	42.0	84	74	3.6	2.04	2.5	476	9.4	41.5	329	1,052	31.3
		7.5	41.0	68	67	4.3	1.57	2.6	491	7.4	44.5	364		34.6
12	10.2	Control	32.5	91	51	5.7	.89	6.1	347	15.4	16.7	97	1,013	9.6
		7.5	29.5	73	60	10.4†	.58	7.5	243	17.7	86.3	355		34.5
13	17.7	Control	48.0	115	112	3.5	3.18	2.2	678	5.8	27.6	312	895	34.8
		10.0	46.5	77	81	3.3	2.44	1.9	412	6.3	55.3	380		42.4
Mean ± S.D.		Control	43.1	116	88	4.8	1.96	3.9	447	12.6	29.9	215		18.5
			±4.6	±19	±28	±2.4	±0.73	±1.3	±140	±5.0	±11	±91		±8.3
		C_6	41.3	77	74	6.1	1.38	3.6	343	10.9	59.3	317		27.3
			±6.9	±12	±13	±2.6	±0.69	±1.7	±113	±3.6	±18	±76		±8.8
Mean Difference			−1.8	−39	−14	+1.3	−0.58	−0.3	−104	−1.7	+29.4	+102		+8.8
P			<0.02	<0.01	<0.05	<0.05	<0.01	>0.5	<0.01	>0.1	<0.01	<0.01		<0.01

* Abbreviations: C_6—Hexamethonium bromide; Hct—Arterial hematocrit; MAP—Mean arterial pressure; A-MV O_2 diff.—Arterial-mixed venous oxygen difference; CO—Cardiac output; TPR—Total peripheral resistance; A.U.—Arbitrary unit = MAP/CO (ml. per sec.); ESBF—Estimated splanchnic blood flow; SVR—Splanchnic vascular resistance; SMCT—Splanchnic mean circulation time; SBV—Circulating splanchnic blood volume; TBV—Total blood volume; S.D. = Standard Deviation.

† The unusually high values for arterial-mixed venous oxygen difference and low values for cardiac output in these dogs are possibly attributable to circulatory inadequacy arising from inapparent infection. Elimination of these figures does not affect the results and does not seem justifiable in any case.

Circulating splanchnic blood volume was determined by the regional dilution technique (10). After rapid injection of approximately 10 to 20 microcuries of IHSA into an external jugular vein, samples of blood were withdrawn simultaneously from the femoral artery and hepatic vein, three times in succession, the first at a slow constant rate of 1 ml. per 30 seconds over a period of two minutes, the second and third at a rate of 2 ml. per 30 seconds during two succeeding 30-second intervals. Approximately 75 ml. of blood was removed from each animal during the entire study. Aliquots of plasma (0.5 ml.) were pipetted onto aluminum planchettes and dried under an infra-red lamp. A commercial detergent (Haemosol®) was used to insure even spreading and to prevent sublimation of iodine. Radioactivity was assayed by means of a Geiger-Muller end-window tube and conventional scaler. Splanchnic blood volume was calculated by dividing the amount of IHSA activity dispersed in the splanchnic vasculature at equilibrium by the equilibrium concentration as follows:

$$SBV = \frac{(\bar{A} - \bar{V}) \cdot t}{[Eq]} \times ESPF \times \frac{1}{1 - Hct.}$$

SBV = Splanchnic blood volume (ml.)
$\bar{A}$ = Mean arterial plasma activity during equilibration (counts per ml.)
$\bar{V}$ = Mean hepatic venous plasma activity during equilibration (counts per ml.)
t = Equilibration time in seconds
ESPF = Splanchnic plasma flow (ml. per sec.)
[Eq] = Plasma activity at equilibrium (counts per ml.)
Hct. = Arterial hematocrit

When equilibrium (or agreement between radioactivity in the arterial and hepatic venous samples within the analytical error) was achieved in the second sampling period, mean arterial activity was calculated as follows: $\bar{A} = (A_1 \times t_1) + (A_2 \times t_2) \div t_1 + t_2$ where A_1 and A_2 are the activities of the first and second samples, respectively, and t_1 and t_2 are the respective time intervals during which blood was withdrawn. With equilibrium in the third period, the values for A and t for that sample were also incorporated in the calculations. The values for mean hepatic venous activity ($\bar{V}$) were computed similarly. Mean splanchnic circulation time was calculated from the values for SBV and ESPF. In four additional anesthetized dogs (9.1 to 15.9 Kg.) pressure tracings (Statham pressure transducer) were obtained successively (within 30 seconds) from the right atrium, an occluded hepatic vein (11, 12), the portal vein, and femoral artery, before and after administration of hexamethonium (0.6 to 0.8 mg. ion per Kg.). Portal venous pressures were measured by means of a polyethylene catheter passed after laparotomy through one of the splenic vein radicles approximately 10 cm. towards the portal vein. All values for venous pressure were measured from a zero reference point located halfway between the dog's back and xiphoid process.

TABLE II

Effect of hexamethonium on mean arterial, splenic venous, wedged hepatic venous and right auricular pressures in dogs *

Dog No.		MAP mm. Hg	Splenic venous pressure mm. Hg	Wedged hepatic venous pressure mm. Hg	Right auricular pressure mm. Hg
14	Control	127	8.0	8.9	1.3
	C_6	85	8.5	7.9	2.0
15	Control	128	12.4	8.5	1.6
	C_6	92	7.9	7.7	1.7
16	Control	120	11.8	8.4	3.2
	C_6	90	9.4	8.3	3.4
17	Control	145	11.6	13.3	−0.4
	C_6	109	11.8	12.6	−4.6

* MAP—Mean Arterial Pressure; C_6—Following administration of hexamethonium bromide.

RESULTS

Comparison of the results obtained in splenectomized and intact dogs failed to show statistically significant differences with the exception of the control mean arterial blood pressure which was significantly lower on the average in the splenectomized animals (102 mm. Hg) ($p = 0.02$) than in the intact dogs (125 mm. Hg). Consequently, the data for all the animals have been combined, and the mean figures, differences between means, and significance of the differences for the group as a whole are presented in Table I.

Arterial and venous pressures

The values for mean arterial blood pressures (MAP) presented in Table I are those obtained at or close to the time of measurement of splanchnic blood flow and cardiac output. The pressures were identical at the time of both measurements throughout each study, save in Dog 9, in which the value was 61 mm. Hg during the determination of splanchnic blood flow and 10 mm. Hg during the measurement of cardiac output following administration of hexamethonium. Mean arterial pressure fell in all dogs by 39 mm. Hg on the average ($p < 0.01$) or 34 per cent. Right atrial, wedged hepatic venous and splenic (or portal) venous pressures did not change significantly (Table II—four animals only).

Cardiac output (CO—Table I)

Cardiac output fell in the 11 dogs in which the measurement could be made. The fall was associated with a decrease in total oxygen consump-

tion (by 14 ml. per min. on the average, $p < 0.05$) and an increase in arteriovenous oxygen difference (by 1.3 ml. per cent on the average, $p < 0.05$). The average decrease of 0.58 liters per minute (or 30 per cent) was statistically significant ($p < 0.01$).

Total peripheral resistance (TPR—Table I)

Total peripheral resistance was calculated in arbitrary units (A. U.) by dividing the mean arterial blood pressure by the cardiac output in ml. per second. Total peripheral resistance did not change on the average ($p > 0.5$) since cardiac output and mean arterial blood pressure decreased to approximately the same extent. It is possible that the marked reduction in TPR which was observed in Dog 9 may be accounted for at least in part by the failure to obtain a value for mean arterial pressure at precisely the time of the measurement of cardiac output.

Estimated splanchnic blood flow and resistance (ESBF and SVR—Table I)

Splanchnic blood flow decreased in 10 dogs, remained unchanged in one (Dog 7), and rose in two (Dogs 4 and 11). The average decrease in ESBF per 10 Kg. body weight was 104 ml. per min., a significant reduction ($p < 0.01$). The splanchnic vascular resistance (SVR—Table I) was computed in arbitrary units (A. U.) by dividing the mean arterial pressure by the splanchnic blood flow (ml. per second). Splanchnic resistance decreased in 7 dogs (Dogs 1, 2, 4, 6, 7, 10 and 11), increased in 2 (Dogs 9 and 12) and remained unchanged in 4 (Dogs 3, 5, 8 and 13). The average reduction in resistance (1.7 A. U.) was not statistically significant ($p > 0.1$).

Splanchnic blood volume (SBV—Table I)

Splanchnic blood volume averaged 215 ml. per 10 Kg. body weight during the control periods, amounting to 18.5 per cent of the total blood volume (SBV/TBV—Table I). After hexamethonium, SBV increased in 12 dogs and remained unchanged in one (Dog 3). The average increment of 102 ml. per 10 Kg. body weight (48 per cent) was highly significant ($p < 0.01$). This change was reflected in an increase in the mean splanchnic circulation time (SMCT—Table I) from 29.9 seconds on the average during the control periods to 59.3 seconds following hexamethonium ($p < 0.01$). The tendency for the hematocrit to fall (Hct.—Table I) could be explained largely on the basis of the withdrawal of blood samples, and it was therefore assumed that total blood volume remained unchanged. On this assumption, the splanchnic vasculature appeared to contain 27.3 per cent of the blood volume during the hypotensive phase.

DISCUSSION

Autonomic blockade following the intravenous administration of hexamethonium produced a fall in blood pressure in dogs that could be ascribed solely to a reduction in cardiac output, in agreement with the results of other workers (1–4). The splanchnic blood flow tended to decrease in proportion to arterial pressure, whereas circulating splanchnic blood volume appeared to rise. The seemingly paradoxical expansion of the splanchnic bed to accommodate a larger volume of blood without significant simultaneous vasodilatation to permit a relatively larger minute flow could be construed as evidence that hexamethonium reduces splanchnic venous tone without affecting the arterioles. Retarded venous return as a result of "pooling" in the splanchnic reservoir—and presumably also in veins elsewhere in the body—could therefore play an independent role in reducing cardiac output and arterial pressure. Further analysis of the data, however, does not sustain the validity of these inferences nor of the conclusion to which they tend—that the major site of action of hexamethonium lies upon the venous side of the circulation.

As a matter of fact, interference with arteriolar activity by hexamethonium is evident in the absence of the usual change (13) in the peripheral vascular resistance during the development of arterial hypotension. The tendency for maintenance of arteriolar cross-section by so-called "basal tone" (14) in vascular beds that have been completely denervated by surgical methods may account for the apparent absence of peripheral arteriolar vasodilation. The difference between the arteriolar and venous responses may actually reflect differences in the distending forces exerted by intraluminal pressures which are inversely related to di-

ameter and are consequently much more powerful in the veins than in the arterioles and capillaries. In this view, the splanchnic arterioles and veins are more or less equally affected by hexamethonium; but in the latter the "basal tone" [as defined by Folkow (14)] is not sufficient to resist distention.

The anatomic evidence (15) suggests that the hepatic and portal venous systems contain the bulk of the circulating blood in the splanchnic bed. If the splanchnic vascular reservoir simply serves as a passive elastic receptacle, its volume would be a function of the distending pressure alone. The fact that SBV increased without change (or with a fall) in the portal venous pressure seems to point to an active role of the venous musculature in determining capacity. Splanchnic blood flow and splanchnic venous pressures decrease similarly in dogs following hemorrhage, but with a reduction, rather than an increase, in circulating splanchnic blood volume (16). The quantity of blood within the splanchnic vasculature therefore appears to vary independently of local pressure differences and blood flow. It seems reasonable to conclude that change in capacity is determined largely by an active dimensional change presumably arising from contraction or relaxation of the smooth muscles in the walls of hepatic and portal venous radicles under the control of the autonomic nervous system.

Estimation of the splanchnic blood volume by the regional dilution technique requires the assumption that I^{131}-labelled human serum albumin is evenly dispersed throughout the bed during an equilibration period of a minute or less. The tracer has little time to find its way into blood not actively a part of the circulating volume and the blood in the splenic pulp is thus not readily accessible to measurement. The change observed following administration of hexamethonium was therefore the same in intact and splenectomized animals.

Owing to the uncertainties regarding the relationships between venous and atrial pressures and cardiac output, it is difficult to evaluate the cardiac effect of hexamethonium or to define with precision the part played by "splanchnic pooling." Unfortunately, the methods currently available are too crude to permit quantitative appraisal of the course of events during the brief interval required for circulatory adjustment. When measurements could be made, a *quasi* steady-state had developed in which cardiac output was persistently depressed in association with a right atrial pressure which had apparently been stabilized at the control level. Changes in pressure too small for measurement may have been implicated as the basis for the continued reduction in output, but the possibility that hexamethonium actually interfered with cardiac function cannot be eliminated.

SUMMARY

The systemic and splanchnic hemodynamic effects of hexamethonium (0.6 to 0.8 mg. hexamethonium ion per Kg. body weight) were studied in thirteen anesthetized dogs—in five at least two weeks following splenectomy. Cardiac output was measured in 11 dogs by the direct Fick method, splanchnic blood flow by the Bromsulfalein method, and the circulating splanchnic blood volume by the regional dilution technique. Femoral arterial, right atrial, wedged hepatic venous, and portal venous pressures were measured directly in four animals.

Arterial pressure decreased in all the animals, by 39 mm. Hg on the average; whereas atrial, hepatic venous, and portal venous pressures did not change. Cardiac output fell, by 30 per cent on the average, to approximately the same extent as the arterial pressure. Estimated splanchnic blood flow decreased in 10 dogs, remained unchanged in one and rose in two; on the average falling significantly ($p < 0.01$) by 104 ml. per min. per Kg. Splanchnic vascular resistance was not significantly affected on the average, but the circulating splanchnic blood volume increased in twelve dogs and remained unchanged in one, rising on the average by 102 ml. per 10 Kg. body weight or 48 per cent.

These findings may be interpreted as evidence for the view that autonomic blockade by hexamethonium causes a fall in arterial pressure by simultaneously reducing cardiac output and interfering with compensatory peripheral arteriolar adjustments. The fall in cardiac output may be attributed to a decrease in venous return as a result, in part at least, of "pooling" in the splanchnic vasculature secondary to diminished venous "tone." Autonomic blockade therefore appears to affect both arteriolar and venous activity.

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PROCEEDINGS OF THE FORTY-NINTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 6, 1957

PRESIDENTIAL ADDRESS

THE TRADITION OF SCIENTIFIC CRITIQUE

BY STANLEY E. BRADLEY

The American Society for Clinical Investigation upholds an old and revered tradition of service to the ideal of a scientific medicine. Within the fabric of our art the threads of learning, skill, and humanity are inseparably woven. When any one of them is lacking or unduly emphasized, the texture is weakened and defective. Unfortunately, throughout most of recorded history, medical science—so called—has been a ridiculous mixture of error, empiricism and superstition. The great physicians, from Hippocrates to Sydenham, held fast to the view that skill and humanity are both best served by an exact and unclouded knowledge of living processes, that medicine is ideally a science to be cultivated by thoughtful investigation. This tradition could not bear fruit, however, until scientific medicine became a realizable possibility with the discovery of the scientific method in the fourteenth and fifteenth centuries (1). Almost immediately civil and ecclesiastical authority proscribed the new way of thinking in the universities (2-4) and for a moment seemed to threaten its development. At this point a new and unexpected element entered the picture.

Scholars and men of affairs, drawn together by a common enthusiasm, formed learned societies to defend and exploit the new method (5). By adherence to the highest possible standards of excellence in work and conduct, by submission to an objective and balanced critique, and by setting an example of intellectual probity, these associations exerted a remarkably pervasive influence in promoting the rise of modern science. The creation of a scientific medicine was defined as a prime objective of the new movement from the very beginning (1, 4, 5). Medical men figured prominently (indeed proved essential) in the conception, gestation, birth, and growth of science (1) but the profession at large was slow in realizing and profitting by its benefits. Then, almost explosively, in the course of a single century, scientific medicine became an overwhelming reality. Again the schools were reluctant to accept the new ideas, and again scientific societies, having no official academic connection —in this country, the Association of American Physicians and the Young Turks—were needed to promote the revolution in medical teaching and practice. Our Society may take its place in the ranks of the older organizations not only because it maintains the same tradition of a scientific critique but also because its vicissitudes and problems are similar.

The idea of the learned Society had its inception in the activities of a variety of lay organizations—luncheon clubs, fraternities, mystical brotherhoods and the like—all concerned with an exploration of the new ideas generated by the rejection of ancient authority. Out of these emerged the first organized scientific society, the Accademia del Cimento, founded in Florence three hundred years ago in 1657 by the Medici family (5). North Italy had been a "forcing bed" of the method and it is not surprising that during the 10 years of its existence the Florentine Academy was able to attract a remarkable group of men whose publications provided a kind of laboratory manual for the development of early scientific investigation. The English were not far behind the Italians and they built more lastingly. In fact, the Italian organization was patterned upon a Utopian research institute sketched out by an Englishman—the "House of Solomon" in Francis Bacon's *New Atlantis*. Bacon's program also formed the basis upon which the Royal Society of London was incorporated in 1660 (6). Academies of science sprang up in France and Germany under the same influence.

The Royal Society survives today as a vital and vigorous institution despite a long history of typical ups-and-downs (6). Approximately a third of the founding members were professionally trained and intellectually disciplined scholars; the remainder were political, military and even ecclesiastical notables with an avocational attitude rather than a primary dedication to the new science. This division persisted for years—at first beneficially, later with detrimental effect. The prestige resulting from the patronage of the Crown, the nobility and men of wealth did much initially to enlist popular sympathy and support. The amateurs provided an enthusiastic and intelligent audience but unfortunately they brought ridicule and derision upon the Society. Their extravagant speculations and absurd preoccupations gave substance to the scoffers' claim that learning produced a gullible crack-pot interested only in the exotic. Strong leadership and the work of the more critical members tended to counteract this picture during the early eighteenth century but by the end of the century the Society had again fallen into disrepute. The non-professional element had tended more and more to regard the organization as a social club, nomination was tantamount to election, and scientific ability did not weigh as heavily as social position and religious views in determining membership. To make matters worse, new, apparently competitive, scientific organizations began to appear; on the one hand, devoted to the cultivation of special fields such as botany and geology and, on the other, concerned

with the general political and social interests of all scientists, as typified by the British Association for the Advancement of Science. With the growing complexity of mature scientific disciplines, a professional attitude of responsibility, stability, and dedication to a stern critique was needed for continued progress. Reorganization of the Royal Society in the 1840's with the establishment of a membership policy designed to limit the membership and to assure suitable qualifications brought the professional attitude to the fore in its affairs. As a result of insistence upon a high standard of excellence in its membership, publications and other activities, the Society quickly regained a position of leadership and fills today a unique position occupied neither by the special societies nor by the British Association, a position in which it embodies and exemplifies the best in scientific critique.

The parallels between the story of the Royal Society and our own need not be labored. We, too, are faced with the problems posed by an unsettled membership policy, by an ill-defined relationship to other groups, and by pressure for expansion. Throughout its long history the Royal Society exerted a remarkable influence primarily because it set the highest possible standard of excellence. With a falling away from the standard its influence waned, to be re-established only with restoration of the standard at a high level. Our Society also has stood for quality, for the highest possible standards of excellence in membership, in the Journal and in the papers presented before this meeting. We can celebrate the tercentenary of the foundation of the learned Societies in no more fitting way than by rededicating ourselves to that ideal.

To the best of my knowledge no conscious plan was ever laid down to attain our Constitutional objectives, but the size and interest of the audiences we are privileged to entertain each year suggest that the Society may have been successful in spite of itself. Medical education has undergone an extensive revision in the years since the first meeting and there is good reason to believe that many members played a major role in the process. In time the triumph of the ideals to which those men subscribed gave lustre to the Society. The standards they established in selecting members reflected great credit upon and gave distinction to the fact of membership itself. The Journal benefitted as a result of the conspicuous quality of the papers it was able to attract. The conscious emulation of the accomplishments of the founding members has done much to promote "the cultivation of clinical research by the methods of the natural sciences" throughout this country and to encourage "the diffusion of a scientific spirit."

Of even greater importance has been the change in the practice of medicine during these years. There is little doubt that now science and practice are closely blended, a result born of dire and urgent necessity. In no other group of professional workers is the scientific attitude more vital or more meaningfully a matter of life and death. Practice today is truly clinical investigation, demanding at once a vigilant critique, a full understanding of the human situation, and a knowledge of fundamental scientific techniques. As it strives to assist in maintaining a high standard of excellence in clinical research, the Society fully serves the ends for which it was founded.

No standard that lacks a divine sanction is readily acceptable; resentments, jealousies, fears, a host of varied considerations combine to raise up an active opposition to it. In each generation the intellectual standards, which professional men live by, must be tested and reaffirmed. This fact was abundantly confirmed in the history of the Royal Society and we should not be surprised that we also must face up to what we are apt to call the "anti-intellectualism of our time," as if it were a problem uniquely ours. Today we are confronted by a widespread assault upon science in medicine. Lip service is paid to its rewards, but an astonishing effort is expended in traducing it. The "scientific spirit," for which this Society stands, is said to produce a cold disinterested character, to dry up the milk of human kindness, whereas a training in social and emotional welfare at the expense of scientific discipline instills by some magic all the human instincts. This is essentially an attack upon standards, an attempt to condone amateurism by redefining it, an effort to make the easy way the right way. I, for one, cannot accept the implication that thought and feeling are incompatible, that the emotional response can somehow supersede, and even take precedence, over an intellectual evaluation. As long as we accept the principles upon which this Society was founded, I think we are all committed to a balanced criterion in which truth and spirit are interlocked; an unattainable ideal perhaps but one vital to progress. The tendency to dissociate feeling and intellect has disastrous effects upon judgment. It provides an excuse to dispense with critical integrity; to measure our friends and our own interests against a different standard than the one we use for outsiders, or to take the easy road of approving of everything and every one in a wonderful world. Unhappily, an unfavorable decision is wounding and difficult of tactful explanation, obviously subject to error, often creating the false impression of intellectual arrogance.

Criticism of the Society is especially acrid in connection with the choice of new members. In eight years of work on the Council of this Society I can say with pride that I have never seen any consideration other than merit take first place in determining nomination though geographical discrimination is often urged upon us. At present the membership is distributed through the land in approximately the same proportion as the population at large. The Western states are somewhat favored in the selection of our officers since every President appoints a nominating committee of three, one each from the Eastern, Central, and Western states and the three nominees selected are usually distributed in the same manner. In this way some 14 per cent of our membership can control one-third of the official positions. This would seem to be a wholly justifiable method of giving encouragement to the West but merit alone should determine our selection of candidates for membership. More recently there has been pressure to increase the membership on the assumption that the number of new

members should be determined by the number of men nominated. The history of the Royal Society shows the fallacy and danger of this assumption. If we wish to allow for the growth of the research establishment in this country, we might gear membership to the number of workers listed in American Men of Science, or better yet, to the number of subscribers or contributors to the Journal of Clinical Investigation. Certainly the number nominated is a poor criterion of anything but the prestige of the Society.

We come now to the scientific session. The papers selected for presentation here have been picked from more than 200 submitted. An honest effort has been made to take the best from a group of excellent titles without reference to subject, geography, or personalities. This is not an easy task; it is certainly never discharged to everyone's satisfaction. Nor are these easy papers. Medicine is a hard and demanding mistress today, always on the move, always changing her focus and concentration. What we learn today will tomorrow seem commonplace, foolish, or obsolete, but now it is new and difficult. As long as progress is being made this must be so; there is no advantage in not facing the fact and swallowing the bitter broth of novel and complex concepts. We must seize upon our facts where we find them, whether by the bedside, at the laboratory bench, or in the animal quarters. In its broadest possible sense clinical investigation is as deeply concerned with the metabolism of the viral particle or animal cell as it is with the behavior of the whole man. Every fact, concept, or method that may have some ultimate bearing upon the problems that face us as physicians should have a hearing in this Hall.

We perpetuate a long and proud tradition that goes back at least 300 years, an intellectual tradition of undaunted critique and of adherence to a high standard of excellence. In these yearly rites of Spring we pay tribute to that tradition and to the view that medicine is a scholarly profession steeped in mercy and dependent upon a devoted skill. To be effective, head, heart, and hand must work as one.

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ABSTRACTS

Cardiorespiratory Effects of Epinephrine Inhalation in Normal Subjects and Patients with Chronic Pulmonary Emphysema. JAMES K. ALEXANDER, JUNICHI MISE, EDWARD W. DENNIS, and ROBERT L. HERSHBERGER, Houston, Tex. (Introduced by James A. Greene).

Observations were made in 5 normal subjects and 12 patients with chronic pulmonary emphysema. Both groups showed increases in pulmonary ventilation, tidal volume, and oxygen uptake, with no significant changes in respiratory frequency, physiological dead space to carbon dioxide or respiratory change ratio. No significant change in arterial blood oxygen saturation or pH occurred, but arterial CO_2 tension fell in the emphysematous patients, suggesting a greater efficiency of alveolar ventilation in this group following the drug.

There was an increase in heart rate and cardiac output in both groups, with reduction in the arterio-venous oxygen difference. Stroke volume and systemic arterial pulse pressure increased or remained the same with no change in mean pressure. Pulmonary arterial mean pressure rose in all the normal subjects and the majority of patients with emphysema, associated with an increase in pulmonary arterial pulse pressure. Calculated vascular resistance in the systemic circulation fell. Pulmonary vascular resistance was unchanged in the normal subjects and the majority of the emphysematous patients, but fell significantly in 3 patients with severe disease. The fall in pulmonary vascular resistance in these 3 patients was not correlated with change in intrathoracic pressure variation, arterial blood oxygen saturation or pH.

Observed increases in pulmonary arterial wedge pressure and right ventricular diastolic pressure, together with a shortened duration of right ventricular mechanical systole, were interpreted as indicative of a change in the dynamics of ventricular contraction. There was a rough correlation between pulse pressure increase and stroke volume change in the left ventricle following epinephrine inhalation in the emphysematous subjects, but not in the normal group.

Abnormalities of Carbohydrate Metabolism of Erythrocytes from Patients with Paroxysmal Nocturnal Hemoglobinuria. K. I. ALTMAN, H. TABECHIAN, L. E. YOUNG,* M. J. IZZO, M. MURPHY, and G. ROBERTS, Rochester, N. Y.

The possibility of a metabolic parameter in hemolysis of red blood cells (rbc) from two patients with paroxysmal nocturnal hemoglobinuria (PNH) was investigated a) by measurements of spontaneous *in vitro* autohemolysis and b) by studies involving P^{32} labeling of phosphorylated intermediates of carbohydrate metabolism in the rbc.

Addition of glucose (30 mM) regularly caused slight acceleration of hemolysis of defibrinated PNH blood (pH adjusted to 6.9) during incubation at 37 C for periods of one to five hours unless glycolysis was inhibited, as by sodium iodoacetate (25 mM). Inosine caused marked deceleration of hemolysis. In none of these experiments could the effect of the additives be attributed to change in pH of the blood or to an anticomplementary effect, but an effect on other serum components of the "PNH hemolytic system" could not be entirely excluded.

When rbc from PNH patients were incubated with $NaH_2P^{32}O_4$ there was a relatively slow rate of incorporation of P^{32} into 2,3-diphosphoglycerate (2,3-DPG) but the total concentration of 2,3-DPG was greater than normal. The rate of entry of extracellular orthophosphate-P^{32} into rbc was considerably slower in PNH than in normal rbc but was regularly accelerated by addition of NaF (5 mM) to PNH blood. When PNH erythrocytes were prelabeled with orthophosphate-P^{32}, the rate of release of P^{32} into the plasma during subsequent incubation was much slower than the release from normal rbc similarly treated. The rate of release of P^{32} from PNH cells was further reduced by addition of NaF (5 mM).

These findings which in the aggregate are unlike those encountered in any other type of red cell indicate that there are abnormalities of intermediary carbohydrate metabolism in PNH erythrocytes. The role of these abnormalities in producing hemolysis *in vivo* is not yet clear.

Iso-osmotic Level of Freezing Point Depression in Boiled Tissues. JOHANNES W. APPELBOOM and WILLIAM A. BRODSKY,* Louisville, Ky.

Since the hypertonicity previously reported in frozen tissues could have been due to rapid metabolism of the homogenates, or to physical changes of proteins subjected to liquid N_2 and high pressure, present experiments were designed to measure osmotic activity after stopping tissue metabolism by heating. Bits of tissue removed from anesthetized dogs were placed in tared beakers containing water at 98° C. After 30 minutes at 98° C, freezing point measurements were made on supernatant fluid, and tissue osmotic activity was calculated from weights of beakers, water and dry tissues. Whole blood, liver, and kidney were hypotonic to serum by 20, 10, and 10 mOsm per L., respectively. Losses of CO_2 during boiling in whole blood, liver, and kidney were 15, 8, and 14 mM per Kg., respectively. After correcting for CO_2 loss, the freezing point depression of boiled tissue extracts became practically equal to that of serum. To evaluate the effect of dilution on activity coefficients of tissue electrolytes, the supernatant was concentrated until its observed freezing point depression was equal to that of plasma, and F. P. measurements were made at several dilutions. At the dilution range of most experiments, the change of activity coefficient of tissue electrolytes was only plus 3 per cent. These data imply: 1) that tissues are isotonic

with plasma, if it is valid to extrapolate to conditions in the living cell; or 2) that liquid N_2 and crushing may split protein or polypeptide chains in tissues, while boiling prevents splitting or promotes adhesion of such chains. Apart from data on erythrocytes, we are not aware of any other measurements on colligative properties of tissues in direct support of the classic concept of intracellular isotonicity.

Labelling of Leukocytes In Vivo with DFP^{32}. J. W. Athens, A. M. Mauer, Helen Ashenbrucker, and G. E. Cartwright,* Salt Lake City, Utah.

Leukocytes have been successfully labelled with radioactive diisopropylfluorophosphate (DFP^{32}). As a label, this compound has the following advantages: (1) the leukocytes can be tagged *in vivo* and thus the disadvantage of handling the leukocytes under non-physiologic conditions is avoided; (2) after an initial equilibration period DFP^{32} is bound firmly and irreversibly to the proteins of the cell; (3) the label is promptly excreted from the body and not reutilized following the death of the cells; and (4) by the use of a plastic scintillation detector the radioactivity in the leukocytes isolated from a relatively small volume of blood (20 ml.) can be measured simply, accurately and rapidly.

Two mg. of DFP^{32}, containing 200 to 400 μc. of radioactivity, are administered either intramuscularly or intravenously. Thereafter, at appropriate intervals of time, leukocytes are isolated free of platelets and erythrocytes, plated between two plastic "Scintilon" squares and the amount of radioactivity determined. The activity is expressed as CPM per mg. of leukocyte nitrogen.

DFP^{32} has been administered to 14 normal subjects. The resulting radioactivity curve in the leukocytes consists of three components. (1) During the first two days there is a rapid decline in the activity. (2) From the second to about the tenth day, the activity remains relatively constant. (3) Thereafter, the activity again declines and is no longer detectable after 19.4 ± 4 days. Varying the initial dose of DFP^{32} between 1.0 and 4.0 mg. and altering the route of administration do not modify the shape of the curve.

Although interpretation of the complex curves is difficult in terms of leukocyte kinetics, the curves have been quite reproducible in normal subjects. This method should be extremely useful in studying the physiology and kinetics of leukocytes in normal and abnormal conditions.

Salicylates and Thyroid Function. F. K. Austen, M. E. Rubini, W. H. Meroney, and J. Wolff, Washington, D. C., and Bethesda, Md. (Introduced by Henry K. Beecher).

A hypermetabolic state associated with depressed thyroid function can be produced in euthyroid patients with rheumatoid arthritis by the daily administration of 7.5 grams of sodium salicylate. The BMR is raised to plus 30 to 60 per cent, serum cholesterol is depressed, and there is a profound creatinuria. Paradoxically, tachycardia, hyperkinesis, and heat intolerance are lacking, and the patients become lethargic without significant alterations in acid-base balance. The serum protein-bound iodine (PBI) gradually falls an average of 40 per cent over several weeks. The biological half-life of I^{131}-thyroxine is reduced to an average of 4.0 days during treatment, and returns to the pretreatment average of 6.8 days upon cessation of therapy. In contrast to hyperthyroidism and certain other hypermetabolic states, in which the extrathyroidal pool of organic iodine is increased, the pools of salicylate-treated patients are diminished. Thyroidal I^{131} uptakes, at 2, 5, and 24 hours, are depressed an average of 30 per cent below pretreatment values. However, the patients respond normally to exogenous TSH as measured by an increase in I^{131} uptake, PBI, and further elevation in BMR. Rat thyroid slices incubated with sera of salicylate-treated patients (3×10^{-3}M salicylate) show no significant reduction in the capacity to incorporate iodide.

While the increased disappearance rates of labeled thyroxine could, in part, account for the lowered serum PBI, the expected compensatory thyroid hyperfunction fails to occur. A direct action of salicylates on thyroid function was not found by *in vitro* studies, and the capacity of the gland to respond to exogenous TSH is retained. These findings are analogous to those obtained in rats with 2,4-dinitrophenol, and suggest that salicylates not only increase metabolic rate by peripheral action, but depress thyroid function by inhibition of the production or release of TSH.

Relationship of Calcium and Citric Acid to Hyperventilation in Man. D. Robert Axelrod, Brooklyn, N. Y. (Introduced by Robert F. Pitts).

Neither pH change nor increase in protein bound calcium is sufficient to fully explain the decreased activity of calcium induced by hyperventilation. In this study, voluntary hyperventilation for twenty minutes produced arterial pH above 7.50 and pCO_2 of 21 mm. Hg, or less. Frank tetany was not usually produced, but all subjects had marked paresthesias. This magnitude of hyperventilation produced essentially no change in plasma calcium concentration. Plasma citric acid levels rose, paralleled by a fall in inorganic phosphate. Maximal changes occurred 40 to 50 minutes after the start of hyperventilation (20 to 25 minutes after cessation of hyperventilation). Plasma potassium levels did not change significantly. Arterial and venous measurements were made of glucose, citric acid, and inorganic phosphate. There was essentially no change in plasma glucose concentration, or glucose A-V differences. A-V differences for phosphate were small and inconsistent, despite the fall in concentration. Citric acid A-V differences indicated a contribution of citric acid from forearm tissue, and a trend of increased delivery after hyperventilation. Hyperventilation produced by 6 per cent CO_2 inhalation did not alter plasma calcium, citric acid, phosphate or glucose concentrations.

The findings of this study suggest that hyperventilation causes a loculation of calcium by metabolically produced citric acid, and that the changes in citric acid levels are a reflection of changes in intermediary metabolism. The time lag before plasma citric acid reached its maximum may be due to the decreased peripheral circulation during hyperventilation, and to the time required to establish equilibrium between cellular fluid and plasma. The increase in citric acid was as much as 3 mg. per cent, and plasma levels exceeded 5 mg. per cent. This magnitude of increase is sufficient to indicate that calcium is complexed with citric acid in hyperventilation, and this complexing may contribute to the paresthesias and tetany of hyperventilation.

Pulmonary Function in Asbestosis of the Lungs; An "Alveolar-Capillary Block" Syndrome. MORTIMER E. BADER, RICHARD A. BADER, and IRVING J. SELIKOFF, New York, N. Y. (Introduced by Alexander B. Gutman).

While the clinical and roentgenographic features of asbestosis of the lungs have received considerable attention in the past, the physiological abnormalities have not been as well defined. Pulmonary function studies in eighteen patients with asbestosis of the lung indicate that the primary pathophysiological defect is one of "alveolar-capillary block."

In all the patients, pulmonary function was evaluated by conventional tests and in five of these by measurement of the diffusing capacity of the lung for oxygen. All had a history of protracted exposure to asbestos dust and had varying degrees of typical roentgenographic involvement. All had dyspnea on exertion; other complaints included chest pain and cough.

Measurement of lung volumes revealed a well-preserved vital capacity, with moderate reduction in six cases and significant reduction in two. Total lung capacity was not significantly reduced. The ratio of residual volume to total lung capacity was less than 35 per cent in all but two cases. The maximum breathing capacity was well maintained. There was significant hyperventilation at rest and exercise in more than half the patients. The index of intra-pulmonary mixing was normal in all but one instance. Arterial oxygen saturation at rest was greater than 94 per cent in twelve patients, and 93 per cent in six. Following exercise, it was greater than 94 per cent in five patients, 90 to 92 per cent in five, 85 to 90 per cent in four, and less than 85 per cent in three. In five patients, diffusing capacity of the lung for oxygen was measured at rest by the method of Riley and Cournand and found significantly reduced in three; the calculated venous admixture was increased in three patients.

The findings of normal maximum breathing capacity, normal intra-pulmonary mixing, absence of emphysematous changes in lung volumes, arterial oxygen unsaturation on exercise, and impaired oxygen diffusing capacity indicate the presence of an "alveolar-capillary block" syndrome.

Physiology of Anemia in "Hypersplenism." MARIO BALDINI, Boston, Mass. (Introduced by William Dameshek).

Our previous experiments with methylcellulose "hypersplenic" rats, performed with the lactating rat technic, showed that the enlarged spleen produces a myeloinhibitory factor. Newborn rats lactated by hypersplenic mothers developed anemia, whereas anemia did not appear when the "hypersplenic" mothers had been previously splenectomized.

Recent experiments demonstrated that "hypersplenic" rats presented both increased hemolysis and bone marrow inhibition. Hemolysis was extracorpuscular in character and disappeared after splenectomy. Bone marrow inhibition was demonstrated using different types of hemolytic anemias (phenylhydrazine, immune serum) in parallel groups of rats. With equal degrees of anemia the reticulocyte values were always lower in "hypersplenic" anemia than in "pure" hemolytic anemias.

Further investigations were performed in selected human cases of "hypersplenism" in which the bone marrow showed no infiltration with abnormal cells. The rate of red cell production was estimated from the chromium^{51} red cell survival time, a valid estimation when the red cell count is stable.

The results obtained demonstrate that:

1. Increased hemolysis was present and was extracorpuscular in type;
2. Red cell production was increased, but the production index then determined remained in the range of 1.5 to 3, whereas in "pure" hemolytic anemias (autoimmune hemolytic anemia and hereditary spherocytosis) the production index was between 5 and 8;
3. Bone marrow production was never below 1, so that after splenectomy, with the disappearance of the hemolytic factor, anemia also disappeared.

In human "hypersplenism," two factors combine to produce anemia: increased hemolysis and bone marrow depression. The latter is "relative" in character since the bone marrow is shown to be deficient only when peripheral need is higher than in the normal. It is not clear whether "hypersplenism" in humans is related to a factor produced by the cells of the spleen or to the fundamental pathologic process which also causes the splenomegaly.

Exaggerated Natriuresis in Essential Hypertension. DAVID S. BALDWIN, ALBERT W. BIGGS, and WILLIAM H. HULET, New York, N. Y. (Introduced by Herbert Chasis).

Hypertensive subjects excrete more sodium than normotensive subjects in response to the infusion of hypertonic saline. The studies reported here indicate that the exaggerated natriuresis is probably initiated by extrarenal factors.

Observations on renal hemodynamics and on Na, water, and total solute excretion were made in 22 hypertensive and 13 normotensive subjects after fluid deprivation and during the last 20 minutes of one-hour infusion of 2.5 per

cent NaCl at 8 to 13 cc. per min. In normotensive subjects the Na excretion fraction averaged 2.3 per cent. In hypertensive subjects this fraction averaged 7.7 per cent. Exaggerated natriuresis induced by saline does not correlate with change in filtered load. Exaggerated natriuresis was also observed in hypertensive subjects during infusion of PAH and inulin in distilled water (2 cc. per min.), showing it does not depend on sodium or osmotic loading.

Basal Na excretion was comparable in the two groups, *i.e.*, hypertensive subjects are not salt losers. With more advanced disease, the Na excretion fraction is correspondingly increased, maintaining Na balance. During Na deprivation hypertensive subjects conserve Na effectively.

An exaggerated natriuresis, similar to that observed in hypertensive subjects, was induced in normotensive subjects by prolonging the saline infusion to two or three hours (avg. excretion fraction 10.2 per cent). Conversely, exaggerated natriuresis in hypertensive subjects was abolished by salt deprivation (avg. excretion fraction 2.9 per cent).

Similar basal urine osmolalities were observed in hypertensive and normotensive subjects after fluid deprivation. During hypertonic saline infusion $T^{c}_{H_2O}$ was comparable in the two groups. It is inferred that the concentrating mechanism is unimpaired in early hypertensive disease.

The absence of evidence of tubular impairment in hypertensive subjects and the fact that exaggerated natriuresis can be obtained without sodium or osmotic loading and is abolished during Na deprivation suggest that the natriuresis is initiated extrarenally.

The Relationship of the Pituitary to Hyperlipemia of Induced Nephrosis in Rats. PETER BALLY and SHOSAKU NUMA, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

Serum lipids were studied in 10 normal and 10 hypophysectomized male Sprague-Dawley rats weighing from 150 to 280 grams before and at four to six days after induction of nephrotoxic serum nephrosis. They were maintained on a Purina checker diet with white bread and raw carrots as a supplement. The onset of nephrosis was evidenced in all animals by massive proteinuria.

The serum levels of total cholesterol, phospholipid and total protein were determined. Lipoprotein distribution was studied with the preparative gradient density tube ultracentrifugation method of Oncley and Mannick. Albumin was determined by paper electrophoresis.

It was found that (1) beta lipoprotein distribution pattern of rat serum seemed identical to the human pattern as far as the location of protein peak concentration is concerned; (2) there is a marked rise of high density (d:1.029–1.053) and low density (d:$<$ 1.026) beta lipoproteins in the nephrotic stage; (3) that in the rat lipemia is not necessarily a reciprocal function of albumin concentration. Hypophysectomized nephrotic rats with severe hypo-albuminemia developed only mild hyperlipemia as compared to control nephrotic rats with equal depression of serum albumin concentration; (4) preliminary studies indicate that cortisone acetate administered (0.1 mg. per 100 gm. body weight per day, s.c.) to hypophysectomized nephrotic rats for two days prior to sacrifice will result in a partial but not complete restoration of the hyperlipemic response.

Similar studies of the hyperlipemic response to nephrosis in adrenalectomized rats are in progress.

An Attempt to Apply the "Cell Separation Theory" to Renal Hemodynamic and Metabolic Measurements in Intact Man. EARL S. BARKER and JOHN K. CLARK,* Philadelphia, Pa.

Pappenheimer and Kinter suggest that "plasma skimming" may separate red cells and plasma within the kidney. Data from over 50 human clearances and renal vein catheterization seem difficult to reconcile with a major regulatory role for such a process even if "cell separation" occurs. Plasma flow through the postulated "long" circuit supplying the peritubular capillary network was estimated by effective renal plasma flow (ERPF) less a small correction for para-aminohippurate (PAH) filtered in the cell-rich "short" circulation by-passing renal tubules. Total plasma flow (direct Fick) so divided in 26 normal subjects under control conditions averaged 557 and 52 ml. per min. for the "long" and "short" circulations.

Renal oxygen consumption (Q_{O_2}) has been described as flow limited by oxygen supplied the tubules. This concept applied to our Q_{O_2} data appears to require unreasonable hematocrit values (low in the "long" circulation, high in the "short" circulation) and to contradict a highly significant inverse correlation between renal blood flow (RBF) and arteriovenous oxygen difference, demonstrable whether RBF increases or decreases. These data are not incompatible with cell separation itself. One might postulate that the concept of flow limitation of Q_{O_2} applies only to deeper nephrons, while the inverse correlation mentioned occurs in peripheral nephrons where more red cells reach the tubules.

Neosynephrine administration or head-up tilt decreases ERPF while PAH-extraction and filtration rate remain relatively constant. Cell separation could explain each change, but the reasoning invoking multiple compensating effects becomes so complicated that renal vasomotor changes seem a more attractive explanation.

A Non-Renal Effect of Adrenal Cortical Steroids upon Potassium Metabolism. FREDERIC C. BARTTER* and PAUL FOURMAN, Bethesda, Md., and Cardiff, United Kingdom.

When desoxycorticosterone-like steroids are administered, loss of potassium is among the well-documented sequelae. When ACTH and hydrocortisone-like steroids are administered, potassium loss also occurs. Two kinds of study were done in man to determine whether the second type of potassium loss has a mechanism similar to that of the first.

In balance studies, it was shown that the second type, as opposed to the first, regularly appears on the first day, seldom lasts more than a day even when treatment is continued, precedes sodium retention, and is not prevented by sodium restriction. It also precedes loss of nitrogen.

In short-term studies it was shown that the second type of potassium diuresis, as opposed to the first, may be accompanied by a rise in serum potassium. Whereas with desoxycorticosterone-like steroids there is an increase in urinary titratable acidity and ammonium with a fall of pH, with hydrocortisone-like steroids there is usually a decrease in urinary titratable acidity and ammonium with a rise of pH.

Whereas the former phenomenon appears to result from a steroid-induced exchange of potassium and hydrogen for sodium ions at the renal tubular level, the latter appears to result from a release of potassium from cells, with the renal effects secondary thereto.

Hyperlipidemia of Nephrosis. JAMES H. BAXTER,* HOWARD C. GOODMAN, and RICHARD J. HAVEL, Bethesda, Md.

Serum lipids (fasting) were studied in 25 patients, age 1 to 65 years, with the nephrotic syndrome. Total cholesterol (TC), free cholesterol (FC) and phospholipid (PL) were elevated in all cases. Triglyceride (TG) was considerably elevated in 19 cases (with TC 354 to 1522), many of whom had lactescent serum. However, in 6 cases (with TC 460 to 675), TG was less than 200 mg. per 100 ml. and serum was clear. These studies together with chemical studies on lipoprotein fractions separated by ultracentrifugation indicated that the spectrum of lipoprotein abnormalities could be divided into three groups characterized respectively by the following patterns: (1) elevation of the density 1.019 to 1.063 fraction alone (in the six cases with clear serum noted above), (2) elevation of the < 1.019 fraction alone (in cases with very high TG and milky serum), or (3) elevation of both these fractions. The pattern changed under observation in 2 cases. On the whole, the patients of group 1, while definitely hypoalbuminemic, had higher levels of serum albumin than most of the others. TC/PL of the lipoprotein fractions was often abnormal.

During remissions and exacerbations of nephrosis there was generally good inverse correlation between levels of serum lipids and level of serum albumin. To determine whether lipid levels are specifically related to level of serum albumin rather than to other parameters of the disease, three nephrotic patients were given courses of concentrated albumin intravenously. A fall in lipids occurred in all cases, though other changes in the disease state also occurred. During a period of more than 2 weeks in which a normal level of serum albumin was maintained in one patient (of group 1 above), serum lipids remained approximately normal. These observations support the concept that hyperlipidemia (hyperlipoproteinemia) of nephrosis is at least in part secondary to hypoalbuminemia.

The Metabolic Effects of Human and Monkey Growth Hormone in Man. J. C. BECK, E. E. MCGARRY, I. DYRENFURTH, and E. H. VENNING, Montreal, Quebec. (Introduced by J. S. L. Browne).

The diverse physiological effects of growth hormone in animals are well described but data on the effects observed in man are inconclusive. Support for the concept of species differences in the growth hormone was provided by Knobil who found monkey growth hormone to be physiologically active in monkeys. We wish to report the metabolic effects observed in a 13-year-old male pituitary dwarf during the administration of human and monkey growth hormone prepared by Raben. Both preparations of growth hormone resulted in a significant enhancement of nitrogen storage which was evident on the second day of its administration and which persisted after cessation of treatment. During the administration of both preparations of growth hormone there was a retention of potassium, phosphorus, calcium and sodium. The positive nitrogen, potassium, phosphorus and sodium balance was due to a decrease in the fecal as well as the urinary excretion of these substances. There was a fall in fecal calcium but an increase in urinary calcium during the growth hormone administration. A gain in body weight occurred during both periods and was maintained for four days after stopping the human material. There was a significant increase in urinary aldosterone excretion during both periods of growth hormone administration. This was most marked with the human growth hormone and was not accompanied by any alteration in urinary 17-hydroxycorticoid and 17-ketosteroid excretion. Impairment of the glucose tolerance curve was evident after 10 days of human growth hormone administration while no change was demonstrable on the fifth day of monkey growth hormone. The effects of these preparations on the serum electrolytes, calcium, phosphorus, alkaline phosphatase, urea, urinary-alpha-amino nitrogen and urinary vitamin C will be described. Further studies with human growth hormone are in progress.

Kinetics of Reaction Between Insulin and Insulin-Binding Antibody. SOLOMON E. BERSON* and ROSALYN S. YALOW, New York, N. Y.

Employing crystalline beef insulin-I^{131} as a tracer, steady state and transient state kinetics of the reaction between beef insulin and insulin-binding antibody have been studied with antisera from insulin resistant and non-resistant subjects. Insulin and antibody react to form a nonprecipitating complex which is in reversible equilibrium with the uncomplexed species. Steady state studies indicate that insulin is univalent in the reaction but that two distinct orders of antibody binding sites are demonstrable in most antisera. The equilibrium constants are of the order of 10^8 and 10^9 liters per mole, respectively. Transient state studies are in agreement and reveal two single order dissociation constants averaging about 0.005 $min.^{-1}$ and 0.2 $min.^{-1}$, respectively.

The demonstration of cross reactions between beef and pork insulin in these studies has suggested that the two

orders of antibody binding sites observed may be due to different affinities of each of the species specific antibodies for beef insulin.

Maximal capacity for insulin binding rarely exceeded 10 units insulin per liter plasma in antisera from non-resistant subjects but ranged from 80 units per liter to more than 400 units per liter in the sera of four subjects with insulin resistance. These studies suggest an explanation for the so-called "insulin unresponsiveness" manifested by some insulin-treated subjects on the basis of rapid binding of administered insulin by antibody. Furthermore, the tendency of insulin-resistant subjects to develop manifestations of hypoglycemia for a number of days following cessation of high dosage insulin therapy is consistent with the slow dissociation of insulin from the insulin-antibody complex observed in these subjects; the insulin-antibody complex serves as a source of "depot" insulin which is released in significant quantities over a prolonged period of time.

An immuno-assay method for the determination of insulin concentrations in body fluids has been developed on the basis of these studies.

The Effect of the Administration of HN_2 on the Utilization of Plasma Iron in Humans. ALBERT BERTINCHAMPS, Y. KENIS, and HENRY J. TAGNON,* Brussels, Belgium.

The administration of methyl-bis (B chloroethyl) amine hydrochloride (HN_2) in therapeutic doses often produces a transient leukopenia resulting from an injurious effect on the hemopoietic system. However, little or no anemia is produced by a single injection of 0.2 to 0.4 mg. of HN_2 per Kg. of body weight. Therefore, the effect of HN_2 on erythropoiesis may not be apparent from observations of the peripheral blood. This effect was investigated by studying the turnover of plasma iron daily by means of radioactive iron after the administration of 0.2 or 0.4 mg. per Kg. of body weight of the drug. Eight patients with cancer were studied who had never been treated before and had not received blood transfusions.

Plasma iron turnover was normal before the injection of HN_2. After the injection, there was a profound depression of turnover reaching a peak between the third and the fourth days and returning gradually to normal between the tenth and the fifteenth days. The maximum depression was of the magnitude seen in cases of aplastic anemias.

These results show that HN_2 in our patients had a pronounced effect on hemoglobin synthesis although such effect did not necessarily express itself in significant changes in peripheral blood values: these remain normal because the depression of erythropoiesis is transient and the red cells do not have a much shortened life span.

The fact that the maximum effect of turnover takes place on the second to fourth days after a single dose of HN_2 cannot be explained presumably by a persistent action of the drug, since the cytotoxic action of HN_2 is apparently completed within a few minutes. The delay may represent the time interval separating the stage of erythropoiesis on which HN_2 has maximum activity and the stage at which hemoglobin is maximally incorporated.

Factors Affecting the Appearance of Ammonia in the Gastric Juice. S. P. BESSMAN and J. C. STAUFFER, Baltimore, Md. (Introduced by Theodore E. Woodward).

The relative permeability of molecular ammonia in comparison to ammonium ion has been proposed as a major factor in the toxicity of circulating ammonium ion. This phenomenon has also been evoked in studies on the mechanism of the toxicity associated with alkalosis. Since a major factor in all of these calculations is the relative pH on both sides of the membrane, a study was made of the pH, ammonia, and urea nitrogen content of arterial blood and of the gastric juice of patients during the course of intravenous infusion of one millimol of ammonium chloride per kilogram in one hour. A lag was found between the rise in blood ammonia and the appearance of an increase in ammonia of the gastric juice. The increase of ammonia could be correlated with the increase of urea in the blood, confirming the observations of Von Korff on the relation of blood urea nitrogen to gastric ammonia. The additional information derived is that there is no correlation between gastric pH and the appearance of ammonia in the stomach. This was studied by adding sufficient bicarbonate to the stomach contents to maintain neutrality. There was no change in distribution of ammonia between gastric juice and blood. The conclusion is drawn that the ammonia of gastric juice is present solely as the result of urease activity, and is not in equilibrium with the blood.

Iron Enzymes in Iron Deficiency: Cytochrome C. ERNEST BEUTLER, Chicago, Ill. (Introduced by Wright Adams).

It is well known that changes in the fingernails, mouth, and esophagus occur in iron deficient patients, and it has been claimed that they may occur in the presence of normal peripheral blood counts. The symptoms of patients with iron deficiency often are greatly out of proportion to the degree of anemia. They may disappear within a few days after the institution of iron therapy, before any hematologic response has occurred. These observations could be explained by changes in iron-containing intracellular enzymes in iron deficiency, but it is commonly believed that the iron-enzymes are not affected by iron deficiency. The origin of this firmly ingrained concept is difficult to trace, but it may be based on the claim by Hahn and Whipple that "muscle parenchyma iron are inviolate stores of iron which are not drawn upon no matter how great is the emergency due to anemia." Careful quantitative determinations of iron enzymes in the tissues of iron deficient animals have never, to our knowledge, been reported. As a model, we have chosen cytochrome C, because of its biological importance and because it is possible to measure the concentration of this heme enzyme spectrophotometrically entirely independently of hemoglobin. Rats were made iron deficient

by bleeding while receiving a diet that was well balanced except for a very low iron content. Control rats were bled in the same manner and received an identical diet except for the addition of an iron salt. A spectacular decrease in the concentration of both liver and kidney cytochrome C was observed in the iron deficient group, the decrease greatly exceeding the decrease in hemoglobin. The widely held concept of the "inviolate nature" of tissue iron enzymes therefore has proved to be false.

Stimulation of the Bone Marrow: A Clinical Study in Man. H. R. BIERMAN,* K. H. KELLY, R. L. BYRON, JR., H. RATTUNDE, and F. CORDES, Duarte, Calif.

The increasing incidence of hematopoietic depressant states due to radio-activity, medication or of idiopathic origin has emphasized the critical need for data concerning leukopoiesis and, in particular, granulocytopoiesis and methods for stimulating the bone marrow. Previous data have shown that leukopoiesis follows many of the basic physiological laws of erythropoiesis. Consequently the response of the bone marrow to the rapid removal of 11 to 88×10^9 leukocytes was studied in 14 patients with various neoplastic diseases.

Serial bone marrow aspirates initially showed a marked depletion of all marrow elements which persisted for 4 to 24 hours as compared with that of the control samples. Immediately afterwards increased granulocytopoiesis and megakaryocytosis appeared with numerous immature forms and the marrow rapidly became hypercellular. Erythropoiesis was similarly stimulated although the hematocrit was maintained between 38 to 45. Leukopoietic activity persisted for 24 to 96 hours during which time increasing maturation of the leukocytes and erythrocytes was observed and increased numbers of immature megakaryocytes made their appearance on the fourth day. The marrow returned to the control pattern within the next 4 to 7 days. The degree and duration of marrow stimulation appeared to be related to the total number of leukocytes removed. Subsequent leukocyte withdrawals showed greater leukopoietic response than initially.

The events of leukopoiesis, megakaryocytosis and erythropoiesis occurred in a well defined chronological order in normocellular, hypercellular, and leukemic marrows. The abrupt hematopoietic stimulus permitted estimates of maturation times and life spans of the various granulocytic forms in the marrow. The effects of hematopoietic stimulation upon the biochemical and hematologic constituents of the circulating blood were related to the degree of leukopoiesis. The significance of these studies to repopulating the marrow and the chemotherapeutic index will be discussed.

Serum Acid Mucopolysaccharides. ALFRED JAY BOLLET, MARIA W. SERAYDARIAN, and RACHEL E. TURNER, Detroit, Mich. (Introduced by Gordon B. Myers).

Mucopolysaccharide containing uronic acid has been isolated from serum by a method suitable for quantitation. It contains two components staining metachromatically with toluidine blue. Both are hyaluronidase-labile. On paper chromatography, one has mobility in two systems resembling chondroitin sulfate and was found to have a molar ratio of hexosamine to uronic acid of 1.1 to 1. The second has a chromatographic mobility resembling heparin and beta-heparin (chondroitin sulfate B), but differs from both in being hyaluronidase-labile. Further characterization is in progress. No component with the chromatographic mobility of hyaluronic acid has been found in serum, although such material is demonstrable in pleural, pericardial and ascitic fluids. These fluids also contain the two chromatographic components found in serum, although the total concentration of acid mucopolysaccharides is usually lower than in serum obtained simultaneously.

Sera from 34 normal individuals were found to have a mean level of 277 mcg. per cent (S.D. $\pm$ 0.042) acid mucopolysaccharide measured as glucuronic acid (carbazole method of Dische). Elevated levels were found in a majority of 55 cases of rheumatoid arthritis, 7 cases of rheumatic fever and 8 cases of systemic lupus erythematosus. Occasional cases with non-rheumatic diseases also showed elevations. Increased levels were not specific for any disease, but were not related to the presence of inflammation in the manner of an acute phase reactant. Chromatographic studies of sera from patients with several rheumatic diseases revealed the same two components found in normal sera.

The Handling of Erythrocyte Iron by the Reticulo-Endothelial Cell. T. BOTHWELL, W. D. NOYES, and C. A. FINCH,* Seattle, Wash.

The present studies involve the administration of radio-iron-tagged erythrocytes rendered non-viable by storage at 4°C in sodium citrate for 24 to 28 days to normal subjects and patients with pertinent disease. The clearance of these cells from circulation, the change in plasma iron concentration and the plasma iron turnover were subsequently measured. Utilizing a double isotope technique (Fe^{59}, Fe^{55}), the amount of iron remaining in the reticulo-endothelial cell and the amount made available to the marrow were determined. *In vivo* distribution of iron was estimated by external body counting.

Studies with varying loads of non-viable cells indicated a maximal clearance rate from circulating blood of about 0.5 ml. RBC per hour per kgm. body weight by normal subjects. Plasma iron levels rose to saturation within 1 hour but plasma iron turnover remained unchanged. In normal subjects approximately one-third of a 1 mg. per kgm. dose of erythrocyte iron was metabolized by the reticulo-endothelial cells and passed on to the marrow without significant delay while the remainder stayed in a slow-moving pool as cellular stores. There was little effect of prior saturation of the plasma iron on this mechanism and there was no specific body localization of erythrocyte iron, the greatest amount appearing in the liver.

In patients with iron deficiency and in pernicious

anemia responding to therapy there was increase in plasma iron turnover with rapid and complete utilization following infusion of non-viable erythrocytes. In infection and iron storage diseases there was little change in plasma iron levels or its turnover, most of the iron remaining in storage.

Thus the reticulo-endothelial system has a finite capacity to process non-viable erythrocytes. The amount of iron stored or delivered to the plasma depends on the dose administered, the needs of the erythroid marrow and the functional integrity of the reticulo-endothelial cell.

Type Specific Antibody Response to M-Protein of Nephritogenic Streptococci in Glomerulonephritis. A. I. BRAUDE,* MARY BONE, BILLIE WAGGONER, and BETTY HATTEN, Dallas, Tex.

The predominance of type 12 streptococci among patients with acute glomerulonephritis led Rammelkamp to postulate that certain streptococcal types are nephritogenic. If his hypothesis is valid, patients with glomerulonephritis should develop type-specific antibodies to M-protein of nephritogenic streptococci. Studies were therefore undertaken to demonstrate such antibodies in an effort 1) to secure immunologic evidence supporting the thesis that nephritogenic streptococci exist; 2) to investigate the role of nephritogenic streptococci in numerous nephritics from whom nontypable or no streptococci were isolated; and 3) to account for immunity against recurrences of glomerulonephritis.

Type specific antibody was measured against type 12, and the provisional Red Lake type by bacteriostasis, mouse-protection, and complement fixation. Blood was examined from: 1) 54 patients on the Red Lake Indian Reservation two years after recovery from epidemic glomerulonephritis; 2) 56 patients with sporadic glomerulonephritis in Dallas.

The simultaneous performance of three tests disclosed antibody in numerous bloods when one of the three tests was negative. Among sporadic cases, 70 per cent of sera subjected to three tests contained antibody to type 12, and 25 per cent to Red Lake; conversely, 91 per cent of sera from Red Lake Indians contained Red Lake antibody and 30.6 per cent antibody to 12. Among normals, only 9.8 per cent contained antibody to 12 and 7.7 per cent to Red Lake. Type-specific antibody was detected from ten days to 4.5 years after onset of nephritis, but failed to develop if penicillin had been given for acute streptococcal infections. The frequent occurrence of antibodies simultaneously to both nephritogenic types suggested a common antigenic relationship.

These results support the bacteriologic evidence for the existence of nephritogenic streptococci by demonstrating almost universal presence of type-specific antibody to nephritogenic streptococci in persons recovered from acute glomerulonephritis. They also account for immunity to recurrent glomerulonephritis caused by both homologous nephritogenic types and heterologous nephritogenic types sharing related antigens.

Hemodynamic Effects of Quantified Mitral Regurgitant Flow. E. BRAUNWALD, G. H. WELCH, JR., S. J. SARNOFF, and A. G. MORROW, Bethesda, Md. (Introduced by S. S. Kety).

A more precise appreciation of the acute hemodynamic consequences of mitral insufficiency was obtained in the dog by producing it in such a manner that the regurgitant flow could be controlled and measured, while simultaneously measuring effective cardiac output, left atrial, left ventricular and aortic pressures. This was accomplished by permitting blood to flow during systole from left ventricle into the left atrium through an external circuit containing a flowmeter. Regurgitant flows up to three times basal cardiac output were tolerated with minimal alterations in forward flow, aortic, left atrial and ventricular pressures. Such regurgitant flows produced only slight depression of effective left ventricular function curves. Any given increase in regurgitant flow required substantially smaller increments in ventricular filling pressure than did similar increases in forward flow, suggesting that ventricular emptying is more complete in the presence of mitral insufficiency. With any given mitral regurgitant orifice, elevating aortic pressure increased regurgitant flow. It was also observed that the left atrial pressure rise brought about by increasing aortic pressure was a function of the mitral regurgitant orifice size. These latter observations were extended to a study of ten patients with mitral valve disease. During transbronchial left heart catheterization, acute elevation of systolic aortic pressure (35 to 85 mm. Hg) induced by infusions of nor-epinephrine markedly raised the left atrial "v" wave of the three patients in whom clinically significant mitral insufficiency was present. In contrast, this effect was not observed in the seven patients with surgically demonstrated pure mitral stenosis. It is suggested that this difference in response can be helpful in the detection of mitral insufficiency, even in the presence of associated mitral stenosis.

Unilateral Renal Disease in Dogs: Method of Induction and Serial Bilateral Renal Function Studies. NEAL S. BRICKER, HARVEY R. BERNARD, and JAMES M. STOKES, St. Louis, Mo. (Introduced by Robert E. Shank).

Many aspects of the functional capacity of the kidney with chronic Bright's disease remain unexplained due to inability to relate physiologic data to control observations of the same subject. Experiments have therefore been undertaken to induce chronic renal lesions in a single kidney of dogs, preserving the integrity of the contralateral kidney. In such preparations, observations may be recorded on the simultaneous behavior of a diseased and normal kidney exposed to the same stimuli, in a common internal environment and with glomerular filtrates of identical composition.

After a bladder-splitting procedure to provide each kidney with a permanent hemibladder, two control clearance studies were performed in each animal. Thereafter a nephrotoxic aminonucleoside (2-6 dimethyl aminopurine 3 amino d-ribose) was infused into one renal artery (over

approximately 10 minutes) while renal venous blood was diverted to prevent recirculation. Blood volume was maintained by infusion of dog whole blood.

Permanent unilateral depression in renal hemodynamics occurred, varying from moderate to severe in degree. Values in the normal kidney invariably increased and RPF occasionally doubled within 16 hours.

When impairment in GFR was marked both the concentration and the absolute amount of sodium in the urine were greater from the diseased kidney despite its lowered filtered loads. During hypertonic saline infusion the percentage of filtered sodium excreted was considerably greater on the diseased side. When GFR was only moderately depressed, however, the diseased kidney in some instances excreted proportionally less sodium than the contralateral organ.

Bilateral renal biopsies were performed before and after aminonucleoside injections. To date there has been no consistent lesion, although in one animal exhibiting marked functional depression, the kidney became contracted and showed dilated tubules containing casts.

Studies are in progress to evaluate the exact role of the aminonucleoside (as well as other nephrotoxic substances) in the genesis of the renal lesion.

Sensitivity to Buffy Coat—A Cause of "Non-Specific" Transfusion Reactions. T. E. BRITTINGHAM and H. CHAPLIN, JR., St. Louis, Mo. (Introduced by C. V. Moore).

Patients requiring repeated blood transfusions sometimes develop increasingly frequent and severe febrile reactions to the infused blood. The reactions are generally considered to be of "non-specific" origin because of inability to demonstrate contamination of the blood, presence of pyrogens, red cell incompatibility, or sensitivity to the donor's plasma. The authors have repeatedly observed in the serum of such patients the presence of agglutinins against donor leukocytes, and sometimes against donor platelets. Patients have been observed to develop leukoagglutinins coincident with the onset of recurrent transfusion reactions.

The present report describes transfusion studies on a series of five patients exhibiting the coexistence of repeated "non-specific" transfusion reactions and demonstrable leukoagglutinins. Prior to each transfusion the donor's blood was partitioned into two fractions—(a) buffy-coat-"poor" (75 per cent of the total red cells, 65 per cent of the total plasma, < 10 per cent of the total buffy coat) and (b) buffy-coat-"rich" (25 per cent of the total red cells, 35 per cent of the total plasma, > 90 per cent of the total buffy coat). Minimal or no reactions were observed following transfusion of the buffy-coat-"poor" fractions. Characteristic reactions (shaking chills, fever) followed transfusion of the buffy-coat-"rich" fractions. There were many similarities to the reactions which customarily follow the injection of bacterial pyrogens: (a) latent period of 60 to 90 minutes following administration, (b) characteristic chills and fever pattern, (c) immediate transient leukopenia followed by more sustained leukocytosis, (d) evidence of a "dosage" effect, and (e) suggestion of "tolerance" following repeated exposure. No reactions were observed during comparable studies on control subjects.

Buffy-coat-"poor" transfusions have been successfully employed in the management of several seriously ill reaction-prone patients. Methods for preparing buffy-coat-"poor" blood for transfusion will be described.

The authors conclude that the leukocyte is an effective antigen and that isoimmunization to leukocytes is a common cause of recurrent febrile transfusion reactions.

Paper Electrophoresis and Gel Diffusion of Sputum in the Diagnosis of Pulmonary Carcinomatosis; Identification of the Fast Moving Component as Albumin. SAMUEL C. BUKANTZ* and ALINE W. BERNS, St. Louis, Mo.

During our pursuit of immunochemical investigations of asthmatic sputum Warfvinge's observations on electrophoresis of sputum was published (Acta med. Scandinav., 1955, 153, 49). He subjected to paper electrophoresis sputa from 100 unselected cases of bronchopulmonary disease. Only a case of bronchiolar carcinoma revealed great increase of a protein component migrating like albumin. Sputum from a patient under our observation whose clinical course resembled bronchiolar carcinoma revealed a similar component on electrophoresis. Necropsy established her pulmonary nodules as metastatic from a primary carcinoma of the stomach. The incidence of this protein in sputum was then ascertained in a blind study. Sputa submitted to the pathologist for cytology were subjected to filter paper electrophoresis at 150 volts and 7.5 to 8.5 milliamperes for 16 hours in veronal buffer at pH 8.6, ionic strength 0.075. One hundred and four sputa from 58 subjects were studied and large amounts of the fast component detected in 11 cases. Five were proven pulmonary carcinomata. A positive diagnosis was not possible in 6 cases, but all presented pulmonary densities and are being followed. Paper chromatograms of hydrolysates of an eluate of the component yielded the amino acid pattern common to proteins. Gel diffusion with sputa and rabbit antisera to fractionated human serum proteins yielded within 18 hours intense bands of precipitate with the anti-albumin serum. This technique, more sensitive than paper electrophoresis and simpler to perform, is being employed with a larger series of sputa and with cervical mucus. Studies are in progress to ascertain the cellular origin of the albumin, traces of which appear in sputa of some apparently non-carcinomatous subjects. Notable is the absence of the component on electrophoresis of grossly bloody sputa or bronchial washings of non-carcinomatous subjects.

Some Psychopharmacological Effects of Atropine. ENOCH CALLAWAY, III, RAYMOND I. BAND, and JACOB E. FINESINGER,* Baltimore, Md.

The effects of the intramuscular injection of 2-mg. doses of atropine were studied in a series of twenty-one normal male volunteers. The personalities of the sub-

jects were evaluated and they were also studied before and after injections of both atropine and saline. The studies included interviews, questionnaires, the Minnesota Multiphasic Personality Inventory, The Progressive Matrices, the Stroop Test, the Luchens Water Jug Test, and the Gottschaldt Test. The results are described in terms of 1) a peripheral sympathomimetic response, 2) a subjective reaction to the central effects of the drug, and 3) the effects of the drug on the subject's level of awareness. The performance of these subjects indicates that atropine in these doses facilitates the performance of a task requiring a broad focus of attention, and interferes with tasks which demand a narrower focus of attention.

Carbohydrate Metabolism as a Measure of Human Platelet Preservation. EDMUND W. CAMPBELL and EDITH V. LO PILATO, Boston, Mass. (Introduced by Samuel Proger).

The preservation of human blood platelets has been evaluated *in vitro* from study of the carbohydrate metabolism. Control values were compared with metabolic studies on platelets modified by the addition of certain metabolic substrates and by temperature changes.

Platelet-rich plasma was stored in silicone-coated containers at 4° C and minus 16° C; substrates were added before and during storage. Radioactive phosphorus was added to some samples to determine the rate of phosphorus turnover. The platelets were separated, the glycolytic esters extracted with trichloracetic acid, and the nucleotides, sugar phosphates, etc., separated by anion-exchange chromatography. Phosphorus was expressed in micrograms per 10^{10} platelets; radioactivity was determined by direct G-M assay.

The addition of adenylic acid, adenosine, adenosine-diphosphate, and adenosine-triphosphate, apparently preserved metabolic activity, since nearly normal amounts of cytidilic acid, adenosine-monophosphate, adenosine-diphosphate, adenosine-triphosphate, ribose and the sugar phosphates were recovered as compared with control values. A moderate increase in inorganic phosphorus was noted but the total phosphorus and per cent recovery were essentially normal. The addition of bivalent metals (*e.g.*, magnesium, manganese) caused similar effects whereas monovalent metals (*e.g.*, zinc) were much less effective. Thus, the addition of some purine nucleosides almost completely preserved metabolic activity; the addition of pyrimidine nucleosides, nucleotides and bivalent trace metals exerted a similar but less pronounced effect. This is contrary to studies reported on erythrocytes, suggesting that platelets may lack a typical cell membrane or manifest a different response to metabolic substrates. The effect of different additives was not necessarily universal throughout the cycle, nor were identical modes of activation present, but definite patterns were established.

A new approach to the problem of platelet preservation has been presented and current studies indicate that correlated investigation of platelet metabolism, physiologic function and *in vivo* viability may help to resolve this problem.

The Effect of Wine in Decompensated Hepatic Cirrhosis. JOHN CARBONE, VICTOR SBOROV,* RUDOLPH FANSKA, and KATHRYN RINGGOLD, San Francisco, Calif.

Alcoholic beverages in ordinary amounts have not been considered to be hepatotoxic to the normal liver. The use of alcohol even in small amounts, however, has been thought to be contraindicated in all forms of liver disease. Experimental data using human subjects to support this latter concept are not available.

To evaluate the effect of alcohol upon the diseased liver, five patients with advanced decompensated nutritional cirrhosis were carefully studied on a metabolic ward. Each patient was used as his own control. The experiment consisted of three twenty-one day periods. A daily basic diet consisting of 120 grams of protein, 300 grams of carbohydrate and 150 grams of fat was used. In the first study period the basic diet was supplemented with a daily ration of 1,000 cc. of Zinfandel wine (750 calories). In the second period the wine was withheld and the diet was maintained isocaloric with 1,000 cc. of sweetened fruit juice. The wine was again administered in the third period.

Liver biopsies were taken at the beginning of each period and at the end of the study. Serial liver function and water, electrolyte and nitrogen balances were measured throughout the study.

Despite the severe liver disease present at the beginning of the study, the administration of the wine did not seem to interfere with normal and rapid convalescence. The rate of clinical recovery was greatest in the first test period and gradually plateaued thereafter. No acceleration of recovery was noted when the wine was discontinued. The liver biopsies showed a high fat content initially which cleared rapidly with the passage of time and did not seem to be retarded by wine ingestion. All patients made a complete and uneventful clinical recovery.

These data do not support an hepatotoxic effect of alcohol in the presence of an otherwise adequate diet.

Correction by Normal Platelets of Deficient Generation of Thromboplastin in a Family of Hemophilics. R. T. CARROLL and R. R. HOLBURN, Philadelphia, Pa. (Introduced by L. M. Tocantins).

A family of hemophilics has been studied in whom the principal defect in the blood was in the platelets. The defect was observed only in males and transmitted by females. Most of the affected males lived beyond the third decade, had little deformity of joints, and most of their bleeding episodes came only after operative procedures or severe injury. The coagulation time of venous blood was prolonged in glass and silicone tubes; recalcified plasma mixtures at 90 per cent concentration were incoagulable. Clot retraction, bleeding time and platelet count were normal. Prothrombin and Ac-globulin concentrations were slightly decreased, other coagu-

lation factors were unchanged. Platelet cofactor activity of the patients' plasma was greater than normal. Addition of normal platelets restored the coagulation time of the patients' plasma to normal, while equal concentrations of the patients' platelets did not. The patients' platelets and serum were insufficient to induce complete thromboplastin generation in normal plasma. Normal platelets restored to normal the thromboplastin generation system containing patients' plasma and serum. Adding equal amounts of patients' plasma to Hemophilia A or B plasma of moderate grade resulted in normal clotting times. Patients' plasma did not prolong the coagulation of normal plasma. Intravenous injections of 100 ml. of platelet-poor normal plasma, either in single or multiple daily infusions, caused no change in the patients' venous blood clotting time, plasma hypocoagulability or rate of thromboplastin generation. One infusion of 100 ml. of platelet-rich normal plasma (4.4×10^6 platelets per cubic millimeter plasma) caused a 30 per cent rise in the platelet count, and a return of the venous blood clotting time, plasma clotting time and thromboplastin generation to normal limits for at least 3 days.

Renal Handling of Glucose in the Dog. FRANCIS P. CHINARD,* W. ROWLAND TAYLOR, MARY F. NOLAN, and THEODORE ENNS, Baltimore, Md.

Under ordinary circumstances the removal of glucose from the urine is sufficiently complete to prevent the escape into the bladder of any significant fraction of the glucose crossing the glomerular barrier. Evidence is outlined below indicating that glucose is transported across the tubule cell without breakdown and resynthesis of the 6 carbon chain and that glucose does not contribute significantly to renal carbon dioxide production. Injection is made nearly instantaneously into the renal artery of solution containing a reference substance, T-1824 usually, creatinine and 1-C-14 glucose. Blood is collected through a catheter in the renal vein; 30 samples are obtained in a total period of 30 seconds to two minutes. The concentration of each of the injected substances is determined in each sample and is divided by the concentration in the injection mass to permit calculation of the relative recoveries. It is assumed that there is no loss of T-1824 during this single passage through the kidney. In 11 experiments the average recovery of creatinine was 76 per cent (the remainder being recoverable in urine); the recovery of C-14 was 98 per cent (insignificant amounts were found in the urine). That the C-14 activity resided in glucose was shown by autoradiography of paper chromatograms on which glucose had been identified chemically and enzymatically (glucose oxidase). As shown by microbiological and chemical degradation there was no randomization of the radioactivity originally present in the 1 position. In other experiments, renal venous blood samples were obtained anaerobically and the C-14 activity of the carbon dioxide determined. Uniformly labelled glucose contributed less than 0.1 per cent of its activity to the carbon dioxide. Carbon 14 labelled lactate contributed significantly.

Inducible (Adaptive) Stimulation of Staphylococcal Penicillinase In Vivo. SIDNEY COHEN and LIPPMAN H. GERONIMUS, Boston, Mass. (Introduced by Benjamin Alexander).

Penicillin resistance in pathogenic strains of staphylococci is mediated by the enzyme penicillinase. The level of penicillinase activity in different strains appears to determine the degree of resistance to penicillin, as measured by conventional sensitivity tests. However, little information is available as to whether the penicillinase activity of an individual strain is fixed or may be altered significantly by environmental changes comparable to those encountered by the staphylococcus within its animal host.

In earlier *in vitro* experiments, we have shown that staphylococcal penicillinase activity may be increased 10 to 30 fold by exposure of staphylococci to penicillin under conditions allowing little or no bacterial multiplication. This increase in activity requires a complex medium and oxygen and is inhibited by Chloramphenicol or 8-hydroxyquinoline. These properties are consistent with inducible (adaptive) stimulation of penicillinase biosynthesis.

An analogous stimulation of penicillinase activity has now been demonstrated *in vivo*. Renal infections were produced in mice by the intravenous injection of a penicillinase-producing strain of staphylococcus. Half of the mice were given 500 units of procaine penicillin G intramuscularly 36, 48, and 60 hours after infection. Seventy-two hours after infection, the animals were sacrificed. The kidneys were homogenized and the staphylococci were harvested, without opportunity for further multiplication, by differential centrifugation in sucrose and KCl solutions at 0° C and their penicillinase activity assayed. The staphylococci from penicillin-treated animals inactivated penicillin 8 times as rapidly (480 units of penicillin/10^9 staphylococci/hour) as organisms from untreated animals (61 units of penicillin/10^9 staphylococci/hour). Additional experiments gave no indication of selection of resistant mutants to explain these results. This evidence supports the hypothesis that, in animal infections, staphylococcal penicillinase may be increased inducibly by penicillin treatment.

Studies on Kinetics and Mechanisms of Potassium Transfer in In Vivo Skeletal Muscle. HADLEY L. CONN, JR., and JOHN C. WOOD, Philadelphia, Pa. (Introduced by Calvin F. Kay).

The quantitative kinetics and particularly the mechanisms responsible for transcellular potassium transfer in skeletal muscle have been little explored. It has become possible, with the aid of tracer methods, to study kinetics of transfer in *in situ* but isolated muscle. Results obtained under different experimental conditions permit insight into mechanisms involved. In the present studies, dog muscle was perfused with arterial blood containing radiopotassium, and the manner of approach to specific activity equilibrium of the venous blood and the muscle

was analyzed mathematically to give potassium transfer constants. With additional data on non-radioactive potassium in blood and muscle and on inulin space, transfer rates could be computed. Transfer rates were obtained for resting muscle, contracting muscle activated by nerve stimulation, for muscle subjected to decalcification, and for muscle perfused with blood containing high glucose and insulin concentrations. In resting muscle the transfer rate was 0.4 mEq. per Kg. (wet weight) per min. The same value was obtained for contracting muscle, and almost the same in the glucose-insulin studies. However, the latter showed a marked uptake of glucose and potassium by muscle, in a mole ratio of four to one, respectively. Decalcification produced a significant increase in potassium rate constants and transfer ratio. Cell potassium concentration was subnormal.

From these results the following tentative conclusions are drawn. In skeletal muscle 1) the cell membrane effects produced by flow of electrical currents, unlike in nerve, are not a significant factor in potassium transfer, 2) potassium transcellular transfer does not occur in association with glucose transport into the cell, but potassium uptake is increased by hyperglycemia because of subsequent glycogen storage and potassium "binding," 3) calcium deficit permits an increased cellular outflux of potassium compatible with the concept that calcium complexes help to maintain the "charged pore" integrity and thus the electrochemical gradient across the cell membrane.

A Method for Serial Cardiac Output Determinations Applied to the Study of Pregnancy Associated with Heart Disease. ROBERT W. CORNETT and JAMES METCALFE, Boston, Mass. (Introduced by C. Sidney Burwell).

The technique of cardiac catheterization is for practical purposes inapplicable to making numerous serial cardiac output determinations in individual patients.

An alternative method, which is applicable to serial use, consists of using the lungs as an aerotonometer and CO_2 as the indicator gas. The PCO_2 of end-expiratory gas is measured during normal quiet breathing. This represents alveolar and arterial PCO_2. A volume of gas is then equilibrated with mixed venous blood by rebreathing, and its PCO_2 measured. The derived A-V difference of CO_2 tension is converted to A-V difference of CO_2 content. With the measurement of CO_2 production, pulmonary blood flow can be calculated.

The lack of data concerning the cardiovascular adjustments to pregnancy in women with heart disease led the authors to make use of this method for determining cardiac output serially and at frequent intervals in a group of pregnant women with rheumatic heart disease.

Over 100 cardiac output determinations were made during pregnancy and the post partum period on 11 women with rheumatic heart disease. Control studies were made on normal non-pregnant subjects, and on pregnant women without heart disease.

The variability of this measurement in non-pregnant control subjects was less than ± 10 per cent of the mean. Comparable consistency of results was found between consecutive cardiac output determination in pregnant subjects, during the period of pregnancy when cardiac output is known to change only gradually.

In all pregnant subjects studied there was an increase in cardiac output reaching its peak during the third trimester, and decreasing before parturition. The mean increase was less in pregnant cardiacs than in pregnant normals.

It is concluded that, because of the reproducibility of results and ease of application, the method described is useful for making serial determinations of cardiac output.

The Influence of Pitressin® and Osmolar Excretion on Urine Water Concentration in Normal Man. J. D. CRAWFORD,* A. N. CUSHMAN, A. PARISI, and M. L. TERRY, Boston, Mass.

The effect of variations in solute outflow on the ratio of water to total osmotically active solute in urine (urine "water concentration") has been studied in normal water loaded human individuals during infusion of constant doses of Pitressin®. Pitressin® influence on urine water concentration is first observed at 0.07 mU and is maximal at 0.75 mU per m.² per minute. When Pitressin® is administered at rates less than 0.17 mU per m.² per minute, the water concentration of urine progressively diminishes with increasing osmolar output. This relationship is reversed when Pitressin® is infused at any given rate greater than 0.17 mU per m.² per minute. The relations between osmolar output and urine water concentration at each of the Pitressin® doses studied conform to the general equation:

$$\text{Log } \Delta K = a + b \text{ Log } X$$

where ΔK is the difference between observed urine water concentration and the constant K; a is the intercept constant; b, the slope constant and X, the output rate of osmotically active solute in urine. Analysis of data obtained in the absence of Pitressin,® at doses which give maximal renal water conservation and at several intermediate dose levels, indicates that correlation coefficients have maximal and highly significant values when K is equal to approximately 6.5. No trend in values for K is observed with changing Pitressin® dose.

The observations confirm and extend those of DeWardener (Clin. Sc., 1955, **14**, 715) and Wirz (Helvet. physiol. Acta, 1956, **14**, 353) indicating that all urine whether concentrated or dilute, is elaborated from a hypotonic precursor. This precursor fluid appears to have a volume which varies in proportion with excreted solute but a constant water concentration of 6.5 ml. per mOsm. corresponding to the aforementioned value for K. In addition, the observations define normal responsiveness to Pitressin®, permit clinical differentiation between "Pitressin® resistance" and normal responsiveness of limited extent and have important implications with regard to fluid tolerance.

A Study of the Factors Affecting the Equilibrium Between Human Erythrocytes and Human Plasma for Radioactive L-Thyroxine. K. R. CRISPELL,* JOSEPH COLEMAN, and HARRY HYER, Charlottesville, Va.

Thyroxine is bound in plasma primarily to a specific protein (T.B.G.) and secondarily to albumin. Previous *in vitro* studies have demonstrated that human erythrocytes will bind radioactive thyroxine. This binding is inhibited by the presence of plasma and to a lesser extent by human albumin. Radioactive thyroxine which is bound to erythrocytes can be removed by incubation with plasma. This suggests there is an equilibrium between erythrocytes and plasma for thyroxine. Studies by others using kidney and heart support this hypothesis.

Two milliliters of Evans Blue Dye (0.5 per cent) will decrease the uptake of thyroxine by erythrocytes from 75 to 37 per cent but does not inhibit the binding by plasma. Two milliliters of Trypan Blue does not inhibit the uptake of thyroxine by the erythrocytes but does inhibit binding by plasma. The presence of this dye increases the uptake by erythrocytes in the presence of plasma from 9 to 37 per cent. A five per cent solution of glucosamine gives results similar to those obtained with Trypan Blue. Trypan Blue, thyroxine, and glucosamine appear to compete for the same binding points on plasma. This is possibly due to a common side group which could act as a site for chelation with a metal and protein of the plasma. Evans Blue Dye does not contain this side group and does not interfere with the binding by plasma. However, it does interfere with the binding by erythrocytes suggesting that the mechanism of binding by the cell of thyroxine differs from that of the binding by plasma.

This *in vivo* system may offer a simple way of testing substances which alter the equilibrium between cell and plasma for thyroxine. The availability of thyroxine to the cell may depend on factors which shift the equilibrium in favor of the cell.

The Survival, Distribution and Apparent Interaction with Capillary Endothelium of Transfused Radiosulfate Labeled Platelets in the Rat. EUGENE P. CRONKITE,* VICTOR P. BOND, JAMES S. ROBERTSON, and DONALD E. PAGLIA, Upton, L. I., N. Y.

The thrombocytopenic purpura produced by whole body irradiation can be prevented by transfusion of fresh platelets (Cronkite and Brecher). In the present studies, the survival, distribution and fate of platelets transfused into the irradiated thrombopenic rat were studied using the radiosulfur platelet labeling method of Odell *et al.*, and appropriate counting and high-resolution autoradiographic techniques. A potential mechanism of platelet hemostatic activity has been demonstrated. Historadioautographs of tissues of irradiated thrombopenic rats sacrificed one hour after transfusion of "homozygous" platelets in number sufficient to raise the circulating platelets levels from 5,000 to 1.5×10^6 per mm.^{3} strongly suggested that either intact platelets, or radiosulfur-labeled material from the platelets adhered to and lined the thrombopenic endothelium. In rats sacrificed four days after transfusion, the radiosulfur activity was found almost exclusively in the splenic macrophages. The survival times of platelets in the normal and irradiated rats were indistinguishable, approximately four days. Definitive interpretation of the adherence to capillary endothelium at 24 hours of the radiosulfur-labeled material involves mathematical considerations of the resolving power possible with S^{35} beta particles (0.165 Mev). It is our belief that the thrombopenic endothelium has selectively taken up the radiosulfur-labeled material of the platelet, or perhaps the intact platelet. Whether this means that platelets contribute some sulfur-rich macromolecular substance (presumably an acid mucopolysaccharide) that is essential for small vessel integrity, or whether platelets play a more secondary role by initiating fibrin formation at a submicroscopic level is a matter for conjecture and further investigation.

Investigation of the Human Small Intestine by Intraluminal Biopsy. WILLIAM H. CROSBY* and BENJAMIN H. SULLIVAN, Washington, D. C.

An instrument has been developed for obtaining biopsy material from the small intestine of the unanesthetized human. It consists of a capsule 11 by 17 mm. containing a rotating, spring-activated knife which is triggered by suction. The suction also acts to draw a specimen of mucosa through a port in the wall of the capsule before the knife is sprung. When a biopsy is to be taken the knife is "cocked" and the capsule is swallowed. It is held captive on a 2-mm. polyethylene catheter which also serves to transmit suction and to retrieve the capsule after the biopsy has been taken. Because the mucosa is sucked away from the muscularis when the biopsy is taken, the knife does not cut deeply into the bowel wall, and detectable bleeding has not been encountered.

Although the instrument was developed to obtain specimens of jejunal mucosa it is used to take biopsies at different levels from the esophagus to the ilium. Multiple capsules swallowed in tandem are used to obtain simultaneous specimens at different levels or to take successive specimens at the same level during the absorptive process.

A brief motion picture film will be shown to demonstrate the application of the biopsy capsule to problems of clinical investigation and diagnosis. Photomicrographs will also be presented to show normal and abnormal specimens of human intestinal mucosa.

Corticosteroid Binding by a Plasma Alpha Globulin. WILLIAM H. DAUGHADAY,* St. Louis, Mo.

Dialysis equilibrium experiments with normal human plasma and increasing amounts of hydrocortisone labeled with hydrocortisone-4-C^{14} have provided evidence for two independent corticosteroid binding systems. One has a high affinity but low capacity; and the other has a low affinity but high capacity. Earlier experiments have indicated that the low-affinity high-capacity binding protein in human plasma is albumin.

The high-affinity, low-capacity binding system possesses a high degree of specificity for corticosteroids in that many slight structural alterations markedly decrease the ability of the derived steroid to displace hydrocortisone-4-C^{14} and corticosterone-4-C^{14} from the binding protein.

Equilibrium continuous flow paper electrophoresis has been used to identify the high-affinity binding protein. Using a low concentration of corticosterone-4-C^{14} and careful preliminary equilibration with the electrophoretic buffer, migration of corticosterone-4-C^{14} with an alpha globulin was clearly established. Moreover, dialysis equilibrium experiments with plasma protein fractions prepared by the Protein Foundation showed a high degree of hydrocortisone binding by fraction IV-4, which contains alpha globulin.

Calculations of corticosteroid transport by normal human plasma with a hydrocortisone level of 14.2 μg./100 ml. show that 13.7 μg. was bound to alpha globulin, 0.4 μg. bound to albumin, and 0.2 μg. in free solution. With a plasma hydrocortisone level of 61 μg./100 ml., only 16.6 μg. would be bound to alpha globulin, 26.9 μg. would be bound to albumin and 17.5 μg. would now be in free solution.

The plasma from six normal adults possessed comparable corticosteroid binding globulin activity. Notable decreases in corticosteroid binding globulin activity have been found in a preliminary survey of several diseases associated with abnormal plasma proteins, including cirrhosis, nephrosis and multiple myeloma. Increased corticosteroid binding globulin activity has not yet been encountered.

Maintenance of Potassium Excretion Despite Reduction of Glomerular Filtration During Sodium Diuresis. Douglas G. Davidson, Norman G. Levinsky, and Robert W. Berliner,* Bethesda, Md.

It has been postulated that under physiologic conditions the filtered potassium is reabsorbed proximally and secreted potassium is the source of urinary potassium. The mechanism for potassium secretion which has been suggested is an ion exchange of potassium for sodium distal to the site of reabsorption. When the amount of sodium reaching the distal tubule is not a limiting factor in the exchange of potassium for sodium, the rate of potassium excretion should be independent of changes in the amount of potassium filtered.

To test this hypothesis the glomerular filtration rate was reduced in one kidney by an inflatable cuff around the renal artery. The contralateral kidney served as a control. Urine was collected separately from each kidney. Diuretics were given along with isotonic, sodium-containing solutions to assure that an adequate amount of sodium would reach the site of the postulated sodium-potassium exchange.

With reduction of the inulin clearance of one kidney by up to 40 per cent the rate of excretion of potassium by that kidney remained equal to that of the control. Greater reductions of inulin clearance were associated with some decrease in potassium excretion relative to the control kidney.

The data strongly support the hypothesis that under these conditions the filtered potassium makes little or no contribution to that excreted in the urine and that the urinary potassium is derived in major part from secretion by exchange for sodium.

The data also indicate that moderate reduction of glomerular filtration by this technique reduces filtration in all of the nephrons but that decreases in excess of 40 per cent may result in a reduction of the number of functioning nephrons.

Urinary Oxalate Excretion in Relation to Renal Stone Formation. Eleanor F. Dempsey, Boston, Mass. (Introduced by Anne P. Forbes).

Approximately 64 per cent of renal stones contain significant amounts of calcium oxalate. Hypercalcuria apparently is a major cause of many of these stones; a concentrated urine due to dehydration may be a factor in others. The object of this study was to clarify the significance of oxalate excretion. Twenty-four-hour urinary oxalate (Dawson's method) has been measured in 28 urines from 20 normal subjects and in 120 urines from 77 patients with possible disorders of oxalate excretion while on normal mixed diets.

Thirty normal subjects and 31 patients with calcium oxalate renal stones excreted 15 to 45 mg. of urinary oxalate per day. Six of the stone patients had hypercalcuria; 19 had normal urinary calcium excretion. Three other patients with idiopathic hypercalcuria and calcium oxalate stones had urinary oxalate excretions of 50 to 80 mg. per day. A child with multiple recurrent calcium oxalate stones without hypercalcuria excreted 130 to 180 mg. per day. Hypercalcuria without stones was not associated with increased oxalate excretion in two patients with hyperparathyroidism, one with lung cancer without bone metastases, two with idiopathic hypercalcuria, and one with hypoparathyroidism on vitamin D. Two patients with sarcoidosis, hypercalcuria, and calcium oxalate stones excreted 60 to 110 mg. per day. Four patients with sarcoidosis, two of whom had hypercalcuria and one who had a calcium oxalate stone, excreted 17 to 39 mg. per day. Five of six patients with hepatic cirrhosis without renal stones excreted 70 to 80 mg. per day. In extended studies of three subjects on the metabolic ward, constant diets rich in oxalate increased the urinary oxalate excretion to two to four times the value observed on low oxalate diets. Forty grams of gelatin added to a constant diet doubled the urinary oxalate excretion.

Adrenal Steroids and Water Metabolism. Joseph F. Dingman and Rene H. Despointes, Boston, Mass., and New Orleans, La. (Introduced by George E. Burch).

The effect of adrenal steroids on solute-free water clearance (C_{H_2O}) and the release of antidiuretic hormone

(ADH) in response to nicotine and hypertonic saline stimulation of the neurohypophysis was evaluated in 9 normal subjects and 2 patients with Addison's disease during constant water loading studies. Cortisone or hydrocortisone (50 to 100 mg.) given to normal subjects orally prior to hydration or intravenously during study augmented the rate of solute-free water diuresis and elevated the dose of nicotine required to stimulate ADH secretion. Intravenous prednisolone, intramuscular DCA and oral or intravenous fluorohydrocortisone had similar effects in 2 studies with each steroid. In 2 other studies with fluorohydrocortisone, C_{H_2O} was slightly depressed and nicotine antidiuresis prolonged or threshold stimulating dose diminished. Two Addisonians off cortisone therapy demonstrated delayed and subnormal diuretic responses to hydration and enhanced sensitivity to nicotine. Increased water diuresis and elevation of nicotine threshold were evident in one patient given 25 mg. cortisone orally prior to hydration and in both patients given 25 mg. prednisolone intravenously during study. The antidiuretic response to intravenous hypertonic saline and Pitressin® was not altered by adrenal steroids. The increase in C_{H_2O} and nicotine threshold was not correlated with variations in solute or sodium excretion. The observations suggest that extracellular hypotonicity induced by water administration does not completely suppress the neurohypophysis and that adrenal steroids increase excretion of solute-free water by additional inhibition of hypothalamic mechanisms controlling ADH secretion which may not be governed by osmoregulatory influences. In some instances, potent salt-retaining steroids may facilitate ADH release. The delayed diuresis of adrenal insufficiency may be attributed, in part, to persistent neurohypophyseal secretion unopposed by adrenal steroids.

On the Mechanism of Transmission of the Atrial Impulse to the Ventricle. JOSEPH R. DIPALMA,* Philadelphia, Pa.

The commonly observed clinical fact that alterations in P-R interval in the ECG are always accompanied by changes in the contour of the P wave was the background for this investigation. The chest was opened in twenty-five dogs under barbiturate anesthesia and electrodes inserted on the S-A node and in the right atrium in the region of the A-V node. The electrode at the S-A node was recorded as an injury or monophasic potential; at the A-V node as a non-polarizable or diphasic potential. Recording was simultaneous by a twin direct writer and oscilloscope. Information derived from the records was the transmission time from S-A node to A-V node, the relations of the contour of the monophasic to the diphasic action potential and the time sequence of the excitation of the A-V node to the action potentials.

It was found that normally the A-V node fires at a predictable position on the contour of the monophasic action potential. This is always at the break in the recovery curve from slow to fast repolarization. Drugs such as epinephrine which shorten the position of this break in the curve consequently shorten the P-R interval. In contrast, stimulation of the vagus and acetylcholine which eliminate the break in the recovery curve lengthen the P-R interval by shifting the position of firing of the A-V node to the bottom of the curve. In all experiments a change in the P-R interval was always accompanied by a corresponding change in the contour of the atrial action potential. This was represented even in the imperfect rendition of the P wave in the ECG limb leads. Actually the study indicated that the delay normally observed in atrial-ventricular transmission resides in the A-V node itself and is dependent on the peculiar excitable process of nodal tissue.

Drug Driving of the Heart in Man. HAROLD T. DODGE and H. V. MURDAUGH, JR., Durham, N. C. (Introduced by H. O. Sieker).

Others have shown isopropylnorepinephrine increases the force of cardiac contraction in experimental animals. This is a study of the cardiovascular effects of isopropylnorepinephrine in man.

Studies were performed before and during intravenous infusion of isopropylnorepinephrine (1 to 2 gamma per minute) for periods as long as 50 minutes. In eight subjects, three normal and five with heart failure, right heart catheterization was performed with measurement of cardiac outputs, Fick method, and pulmonary arterial pressures.

During isopropylnorepinephrine infusion all subjects showed an increase in cardiac output (mean 37 and 63 per cent from mean basal cardiac indices of 3.40 ± 0.44 and 2.10 ± 0.68 in normal and heart failure groups, respectively) and a decrease in A-V oxygen difference (1.0 ± 0.03 and 3.0 ± 1.63 volumes per cent in the two respective groups). Oxygen consumption remained unchanged. Heart rate usually increased slightly, brachial arterial pulse pressure increased with little change of mean arterial pressure, peripheral vascular resistance decreased, and left ventricular work increased in all subjects. All but one of the patients with heart failure had an increase of left ventricular stroke volume (mean 30 per cent) and stroke work (mean 45 per cent). This was associated with no change or a decrease of pulmonary arterial diastolic pressure. Right-sided filling pressure decreased in two subjects where this was measured.

Renal clearances were performed in three subjects. Urine flow increased in all. G.F.R. remained unchanged in two subjects with normal control G.F.R. values. In the third subject with severe congestive heart failure G.F.R. increased from 30 to 139 ml. per minute.

These studies demonstrate isopropylnorepinephrine has a positive inotropic effect on normal and failing hearts. This occurs whether or not the patient is receiving digitalis, suggesting it increases cardiac contractility through a different mechanism than does digitalis. The cardiorenal responses differ from what is observed when the heart is driven by exercise.

Plasma NEFA as an Index of Carbohydrate Utilization. VINCENT P. DOLE,* EDWIN L. BIERMAN, and THOMAS N. ROBERTS, New York, N. Y.

In normal subjects the concentration of NEFA (non-esterified fatty acids) in plasma falls sharply after administration of glucose or insulin, increases slowly with fasting, and rises to exceptional levels after injection of epinephrine. The present studies suggest that these responses might provide a clinically useful index of changes in carbohydrate utilization. Since plasma NEFA has a half time of only a few minutes, the concentration of these fatty acids responds almost immediately to any variation of turnover rate.

Oral glucose or intravenous glucagon (as exogenous or endogenous glucose load, respectively) was given to normal and to diabetic subjects. With either kind of load the diabetics showed a delayed fall of NEFA, a subnormal amplitude of response and a slow return to baseline. Patients with diabetic acidosis and incipient coma showed a progressive rise in NEFA, preceding the onset of ketonuria, and little response to glucose. Treatment with insulin caused a prompt fall of NEFA, occurring several hours before the disappearance of ketonuria.

Although the NEFA response to glucose load seems to require insulin, the reduction probably is not a direct effect of this hormone since fructose, which can be metabolized independently of insulin, caused a prompt fall of NEFA. The pituitary hormones also appeared not to be primarily involved in the response since a sharp fall of NEFA was produced by feeding glucose to a hypophysectomized patient. Orinase (2 gm.) injected intravenously into normal subjects or stable diabetics caused a fall of both glucose and NEFA. However, when given to juvenile diabetics with histories of repeated ketosis, orinase failed to reduce either glucose or NEFA, whereas insulin caused a prompt reduction of both.

The results to date suggest the hypothesis that utilization of carbohydrate inhibits release of NEFA from fat depots. In the unstable diabetic this mechanism seems to be faulty.

A Dual Vitamin B_{12} Plasma Absorption Mechanism. ALFRED DOSCHERHOLMEN and PAUL S. HAGEN, Minneapolis, Minn. (Introduced by Edmund B. Flink).

Plasma vitamin B_{12} absorption curves have been obtained by a radiometric technique in patients with pernicious anemia with and without intrinsic factor as well as in control subjects after the administration of oral test doses ranging from 0.56 μg. to 500 μg. radioactive vitamin B_{12}.

Patients with pernicious anemia were found to have no or negligible plasma absorption when oral test doses of 0.56 μg. were administered alone. When intrinsic factor concentrate was added, absorption curves were obtained similar to those found in control subjects given test doses in the same range. They were characterized by no plasma radioactivity during the first four hours of the test and a peak concentration in the 8 to 12 hour interval.

Oral test doses of 50 to 300 μg. vitamin B_{12} given alone to patients with pernicious anemia resulted in completely different absorption curves. They were characterized by an early rise in plasma radioactivity and peak concentrations usually 3 to 6 hours after the test. This type of plasma absorption of vitamin B_{12} appears to be independent of intrinsic factor. The addition of this factor abolished the early rise in plasma concentration and resulted in a peak concentration at 12 hours. Although intrinsic factor concentrate inhibited the plasma absorption not mediated through this factor, it enhanced hepatic uptake of radioactivity.

Control subjects given test doses of 200 to 500 μg. vitamin B_{12} revealed a slightly diphasic plasma absorption curve with an early rise in the radioactivity. A test dose of 10 μg. gave a curve similar to those observed with test doses of 0.56 μg., while doses of 50 to 100 μg. revealed intermediate curves.

The dual vitamin B_{12} plasma absorption mechanism lends support to the hypothesis of two distinct gastrointestinal modes for the absorption of this vitamin.

Comparative Analysis of Radioactive Strontium and Calcium Administered Simultaneously to Man. ELIAS C. DOW, WILLIAM H. BAKER, and JOHN B. STANBURY,* Boston, Mass.

When calcium^{45} and strontium^{85} are administered intravenously to man, a larger fraction of the calcium^{45} is found in the bones. In dog and man, renal excretion of strontium^{85} is faster than calcium. It has not been clear whether differences in deposition of calcium and strontium in bone result from differential handling by bone or by kidney, or by both.

A single intravenous injection of strontium^{85} and calcium^{45} was given to several patients with bone disease. For the succeeding three weeks or more, serum, urine, and feces were measured for content of calcium^{40}, calcium^{45}, and strontium^{85}. In all cases the urinary excretion of strontium^{85} was greater than the excretion of calcium^{45}, resulting in at least 30 per cent less body retention of strontium^{85} than calcium^{45} after 21 days. Urinary specific activities of strontium^{85} (per cent administered dose of strontium^{85} per gram of calcium^{40}) were initially 3 to 8 times the urinary specific activities of calcium^{45} (per cent administered dose of calcium^{45} per gram of calcium^{40}) and decreased exponentially. However, the specific activities of strontium^{85} in the serum were equal to those of calcium for one to five days, but then fell to significantly lower values. The specific activity of strontium^{85} in the stools was higher than that of calcium^{45}.

Simultaneous "pool sizes" (per cent of isotope retained in body per specific activity of isotope in the serum) were calculated for successive three-day periods for each isotope. In spite of increased renal and fecal excretion of strontium^{85}, the "pool sizes" of strontium and calcium were virtually the same in each case studied.

The data indicate that renal excretion is a major factor controlling the amount of strontium^{85} available for

deposition in bone, but bone does not discriminate between strontium[65] and calcium[65].

The Production of a Hypernatremia and Hyperosmolarity in the Diabetes Insipidus Dog Infused with Ringer's Solution. LEONARD S. DREIFUS and JEREMIAH STAMLER,* Chicago, Ill.

This study was undertaken to assess the response of the unanesthetized diabetes insipidus (DI) dog to prolonged infusion of Ringer's solution (9 to 11 cc. per minute for several hours). In contrast to the ability of the intact animal to maintain constancy of plasma Na and total solute concentrations during infusion, DI dogs developed a progressive marked hypernatremia and hyperosmolarity. This alteration in plasma was a resultant of the abnormal renal H_2O-electrolyte exchanges in the DI dog. Thus, the DI animal exhibited normal rates of Na and total solute excretion (despite depressed RPF and GFR), but markedly elevated urinary flow rates. Hence, the DI animal elaborated a grossly hypotonic urine, in contrast to the approximately isotonic urine of the intact dog. These responses to prolonged Ringer's infusion were typical manifestations of the derangement in renal function in the DI animal. These findings may be applicable to man: 1. Diabetes insipidus may be differentially diagnosed by demonstrating hypernatremia in response to Ringer's infusion. (This test would obviate need for infusion of distressing hypertonic NaCl solutions.) 2. Diabetes insipidus patients, as well as patients with transient DI due to intracranial disease or injury, should be approached very cautiously when saline infusions are contemplated; during such infusions, frequent sodium and/or total solute determinations are indicated to avoid production of hypernatremic and hyperosmolar states.

Effects of the Formic Acid Analogues of Thyroxine and Triiodothyronine on Cholesterol Metabolism. CHARLES H. DUNCAN and MAURICE M. BEST, Louisville, Ky. (Introduced by J. Murray Kinsman).

Four thyroxine-like compounds have been investigated with regard to their relative effectiveness in preventing thiouracil-induced goitre and in effecting a reduction in serum and liver cholesterol levels. So studied were l-triiodothyronine (T-3), l-thyroxine (T-4), triiodothyroformic acid (TFA-3), in which a carboxyl group has been substituted for the alanine side chain of triiodothyronine, and tetraiodothyroformic acid (TFA-4), the comparable formic acid analogue of thyroxine.

In the control group of 300-gram male albino rats, the addition of 1 per cent cholesterol and 0.5 per cent thiouracil to their diet for 2 weeks resulted in a mean increase in thyroid weight from 6.5 to 18.9 mg. per 100 gm. body weight. The minimum daily dose per 100 gm. body weight of each of the four compounds which prevented thyroid enlargement was as follows: T-3, 0.8 mcg.; T-4, 3 mcg.; TFA-3, 900 mcg.; and TFA-4, 3500 mcg.

The mean liver cholesterol in the control animals was 1162 mg. per 100 gm. liver. Each of the four compounds, administered in approximately the goitre-preventing dose, exerted some inhibitory effect on cholesterol accumulation in the liver. T-3, 0.7 mcg., resulted in liver cholesterol of 663 mg.; T-4, 3 mcg., in mean liver cholesterol of 744 mg.; TFA-3, 800 mcg., in mean liver cholesterol of 615 mg.; and TFA-4, 2000 mcg., mean liver cholesterol of 346 mg. All doses are expressed as mcg. per 1000 gm. body weight per day, and liver cholesterol as mg. per 100 gm. liver. The disproportionately greater effect of TFA-4 on liver cholesterol was also demonstrated at other dosage levels. l-Thyroxine, 6 mcg., twice the goitre-preventing dose, effected a reduction in liver cholesterol to 599 mg. per 100 gm. In contrast, TFA-4 at 4000 mcg., just above the goitre-preventing dose, resulted in liver cholesterol of only 268 mg. per 100 gm., essentially that of the rat fed neither cholesterol nor thiouracil.

TFA-4 has been administered to human subjects. In daily doses of 200 mg., it was found to be metabolically active as evidenced by increased rate of oxygen consumption and decrease in serum cholesterol.

Some Determinants of Urine Composition in Man: Separated Renal Functions in Patients with Unilateral and Bilateral Renal Diseases. HARRIET P. DUSTAN and EUGENE F. POUTASSE, Cleveland, O. (Introduced by A. C. Corcoran).

Glomerular filtration rate changes have been considered responsible for the small urine volume with low sodium concentration characterizing renal artery constriction in the dog and renal artery occlusive disease in man. To investigate this, separated renal functions were determined in thirteen hypertensive patients with unilateral or bilateral renal diseases.

Group I was composed of seven patients with nephrosclerosis; Group II, two with unilateral pyelonephritis; Group III, two with unilateral renal artery occlusive disease; Group IV, two with occlusive disease on one side and pyelonephritis or hydronephrosis on the other. Ureteral urines were collected during mannitol loading and Pitressin® anti-diuresis; standard clearance studies were performed; water and sodium conservations were calculated.

In Group I, the differences in renal plasma flow (RPF), filtration rate (GFR) and filtration fraction (FF) between the two sides were as reported by Chasis and Redish. In six of the seven, urine osmolality (U_{mOsm}), water reabsorption ($T^c_{H_2O}/C_{Man}$), and per cent reabsorption of filtered sodium load (% Na Reab.) were similar. Small GFR differences, in four, were accompanied by parallel differences in sodium concentration (U_{Na}). In Group II, there were marked disparities in RPF and GFR with corresponding changes in urine volume (V), but U_{mOsm}, $T^c_{H_2O}/C_{Man}$, and % Na Reab. were identical. In Group III, RPF, GFR, FF and V were all decreased on the involved side; in one patient, these changes were associated with markedly lowered U_{Na}, increased % Na Reab., U_{mOsm}, and $T^c_{H_2O}/C_{Man}$; in the other $T^c_{H_2O}/C_{Man}$ was, perhaps, significantly increased but U_{mOsm}, U_{Na}, and % Na Reab. were the same on both sides. In Group IV, the kidneys with the artery lesions had higher RPF and

GFR than their mates, with corresponding but not impressive changes in V, yet, in spite of this, U_{mOsm}, $T^c_{H_2O}/C_{Kan}$, and % Na Reab. were increased.

The data suggest that GFR is not, alone, responsible for urine composition in unilateral renal disease.

The Effect of Exposure of Erythrocytes from Acid Citrate Dextrose (ACD) Whole Blood to 5 Grams Per Cent Dextrose and 0.225 Grams Per Cent Sodium Chloride on RBC Size, Potassium Concentration and Content, Glucose Content, and In Vivo Survival in Man. FRANKLIN G. EBAUGH, JR., JOSEPH R. BOVE, and RICHARD S. STEMLER, Hanover, N. H. (Introduced by S. Marsh Tenney).

Exposure of erythrocytes (1 part whole ACD blood to 2 parts dextrose solution) to 5 per cent dextrose and 0.225 gm. per cent NaCl for 3 hours results in 138 ± 5 (134 to 177) per cent increase in hematocrit, 1100 ± 270 (690 to 1660) per cent increase in glucose, and a decrease in potassium *concentration* to 58 ± 5.7 (50 to 63) per cent of normal, but no change in K content per RBC. Three minutes after replacement of the dextrose solution with autogenous plasma the hematocrit was 149 ± 14 per cent that of the ACD whole blood. The hematocrit was corrected for hemolysis occurring in 10/20 of the observations during addition of plasma. After one hour in plasma the hematocrit, RBC glucose, and K concentration returned to normal. The *in vivo* survival 10 minutes after transfusion of Cr^{51} labeled RBC previously exposed to dextrose solution for 3 hours ranged from 5 to 127 per cent the transfused amount and showed no correlation with the hematocrit increase, or the RBC glucose concentration, but did show a significant inverse correlation ($r = -0.565$, $n = 14$, $p < .05$) with the per cent increase in hematocrit after subsequent 3-minute equilibration with plasma. The *in vivo* survival of the same RBC in the same recipient was increased 14 ± 19 per cent ($p < .05$) after equilibration with plasma for one hour. The survival of one part ACD blood and 19 parts of dextrose solution, in contrast to the ⅓ mixture, showed a consistent relationship between survival and time exposed to dextrose solution, the results being 50, 20, 15 and 8 per cent survival after 30, 60, 90, and 120 minutes, respectively. The above data indicate that an increase above 170 ± 10 per cent normal RBC size under the above conditions is incompatible with normal RBC *in vivo* survival, and that exposure to the dextrose solution exerts a slight but significant deleterious effect on RBC survival apart from RBC volume change.

The Pressure-Volume Characteristics of Human Forearm Veins During Epinephrine Infusion. JOHN W. ECKSTEIN and WILLIAM K. HAMILTON, Iowa City, Ia. (Introduced by James W. Culbertson).

Six normal men were studied in the supine position with a plethysmograph on the left forearm, a pressure needle in the right antecubital vein, and an infusion needle in a foot vein. Forearm venous distensibility (VD) was measured plethysmographically (Litter, Wood, Wilkins). Pressure-volume curves obtained by this method originate at the volume existing at an effective venous pressure (EVP) of 1 mm. Hg. Curves are formed by plotting volume changes against increments in EVP from 1 to 31 mm. Hg. The point on each curve corresponding to the local venous pressure (LVP) measured in the unencumbered (right) forearm indicates local venous volume (LVV). Observations on VD and LVP were made before, during and after epinephrine infusion.

During control periods in 6 experiments VD for an EVP of 30 mm. Hg averaged 4.1 cc. per 100 cc. of forearm tissue and ranged from 3.0 to 4.5. When epinephrine was infused at 15.0 mcg. per min. the average value fell to 2.6 cc. per 100 cc. and the range was 1.9 to 3.3. LVP rose during this infusion with increases which averaged 2.9 mm. Hg and ranged from 1.5 to 5.5. During control periods LVV averaged 1.9 cc. per 100 cc. and ranged from 1.5 to 2.5; with epinephrine these values fell to levels averaging 1.4 cc. per 100 cc. and ranging from 1.0 to 1.9.

In 8 additional experiments epinephrine infusions of 7.5 or 10.0 mcg. per min. regularly caused reductions in VD and increases in LVP while inconsistent small changes occurred in LVV.

Under the conditions of these 14 experiments epinephrine infusion uniformly caused venous pressure to rise and venous distensibility to decrease. At an infusion rate of 15.0 mcg. per min. the net effect in each case was a shift of blood out of the forearm veins.

The Relationships Among Serum Sodium Concentration, Serum Osmolarity and Body Composition in Man. I. S. EDELMAN,* J. LEIBMAN, M. P. O'MEARA, and L. W. BIRKENFELD, San Francisco, Calif.

A quantitative relationship between electrolyte content of the body and serum sodium concentration has not been established in previous studies, nor is such a relationship predictable because of uncertainties with respect to osmotic equilibrium across cell membranes and possible mobilization of bone sodium in pathological states. The observations to be presented indicate that serum sodium concentration (Na_s) and serum osmolarity $¶_s$ are linear functions of the ratio of exchangeable body sodium (Na_e) plus exchangeable body potassium (Ke) to total body water (TBW). This ratio $\left[\frac{Na_e + Ke}{TBW}\right]$ is a measure of the proportion of osmotically active cation to total body water.

Measurements of $¶_s$ by freezing point depression and simultaneous Na_s have been performed in 69 patients. $¶_s$ is a linear function of Na_s when appropriate corrections are made for serum NPN and glucose with an S.D. $¶_s/Na_s$ of ± 5 mOsm. Na_e, Ke and TBW were measured in 66 patients by isotope dilution. Thirty-six of these subjects were edematous because of cardiac, hepatic or renal disease. The Na_s varied from 110 to 155 mEq. per L. in these subjects. Neither Na_s nor $¶_s$ showed a significant correlation with Na_e, Ke, TBW, Na_e/TBW or

Ke/TBW. Both Na_e and $¶_e$ showed a highly significant correlation with $\left[\frac{Na_e + Ke}{TBW}\right]$. The covariance of Na_e with respect to this ratio was only ± 6 mEq. per L.

These results support the following conclusions:

a) The primary determinants of serum sodium concentration are body sodium, potassium and water contents and provide the basis for a classification system for hypo- and hyper-natremias,
b) Extracellular and intracellular fluids are in osmotic equilibrium.

Metabolic Interrelations of Calcium and Magnesium in Patients with and without Osteolytic Disease. LEONARD P. ELIEL,* WILLIAM O. SMITH, and JOSEPHINE HAWRYLKO, Oklahoma City, Okla.

Tremors, convulsions and hypomagnesemia of 0.9 to 1.1 mEq. per L. (normal mean 1.60 mEq. per L.) were observed in three patients with osteolytic disease in association with episodes of hypercalciuria, hypercalcemia, and renal impairment. Urine excretion of magnesium varied directly with urine calcium and in twenty-fold greater amount than could be accounted for on the basis of bone destruction alone. Balance data revealed intracellular and extracellular magnesium depletion. When a hypercalciuria of kelated calcium was produced, in one of the patients, with intravenous EDTA, however, no increase occurred in urinary magnesium.

Two patients without osteolytic disease and two with osteolysis, but with normal renal function, were studied by daily intravenous calcium loading while on metabolic balance regimes. The urine magnesium decreased to 20 to 33 per cent of the control values while urine calcium increased by 3 to 70 fold. However, the serum magnesium declined to 36 to 75 per cent of control values; levels as low as 0.66 mEq. per L. were noted. Stool magnesium showed little change. A diuresis of sodium, chloride and water occurred in all patients. Balance calculations revealed movement of magnesium into the cells. None of these patients developed symptoms of magnesium deficiency. Similar changes in magnesium did not occur with infusions of an equivalent amount of sodium.

It appears that the normal metabolic response to intravenous calcium loading is an intracellular shift of magnesium with a resultant decrease in its extracellular concentration and renal excretion. Patients with osteolytic disease and renal impairment show increased renal loss and extracellular depletion of magnesium during hypercalciuria of ionized calcium, presumably due to tubular damage.

Collateral Sensitivity to Methotrexate in 6-Mercaptopurine-responsive Adult Acute Leukemics. ROSE RUTH ELLISON, New York, N. Y. (Introduced by Joseph H. Burchenal).

Certain antimetabolites may produce temporary remissions of acute leukemia. These effects apparently result from blocking nucleic acid biosynthesis at some point. Bacterial and *in vitro* studies have indicated that the folic acid antagonists and 6-mercaptopurine (6MP) act differently, the former inhibiting *de novo* synthesis of purines and pyrimidines and the latter affecting incorporation of purines into nucleic acid.

The folic acid antagonists produce temporary remissions in 35 to 50 per cent of children but are comparatively ineffective in adults (3 partial remissions and 1 clinical improvement in 38 cases). The data to be reported concern 29 adults with acute leukemia who initially responded to 6MP but developed resistance to 6MP and relapsed. They were then treated with the folic acid antagonist, 4-amino-N^{10}-methyl pteroylglutamic acid (Methotrexate). Nine of the 29 showed some degree of response. Three developed complete hematologic and clinical remissions, 3 showed partial marrow but good clinical and peripheral blood effects, and 3 were clinically benefited with some improvement in the peripheral blood picture. This is an incidence of 31 per cent, substantially higher than the 11 per cent observed with Methotrexate alone.

The mechanisms responsible for the initial resistance of some leukemias to therapy, and for the development of resistance in the initially responsive are unknown. The occurrence of apparently increased sensitivity of the leukemic process to Methotrexate in the patients reported parallels findings by others in 6MP-resistant strains of both *L. casei* and L 1210 mouse leukemia. In *L. casei* the increased sensitivity of the 6MP-resistant strain to Methotrexate is associated with an increased requirement for folic acid. The clinical data suggest that either the 6MP-resistant strains of leukemic cells which evolved have pathways for nucleic acid synthesis qualitatively different from those previously utilized, or a Methotrexate-responsive group has been selected out by virtue of its also being responsive to 6MP.

Observations on the Action of Combined Glucagon-Insulin Infusions in Diabetic Patients. HAROLD ELRICK, Y. ARAI, and V. YEARWOOD-DRAYTON, Denver, Colo. (Introduced by Gordon Meiklejohn).

Studies from this laboratory have shown that in nondiabetic patients glucagon and insulin together augment peripheral glucose utilization more than either hormone alone. Furthermore, combined infusions resulted in neutralization of the opposing action of the two agents on arterial glucose levels.

The purpose of this paper is to report on 39 glucagon-insulin experiments in 33 diabetic patients. Under standardized conditions, after an 11-hour fast and 25 hours after the last dose of insulin, glucose (142 to 185 mg. per min.) and insulin (3.3 to 6.5 γ per min.) were administered by constant intravenous infusion. Crystalline glucagon (1.1 to 5.1 γ per min.) was added 55 or 70 minutes following the start of the infusion. Arteriovenous samples (16 pairs) were obtained throughout a two and one-half-hour test period in 16 patients. A-V/A values (arteriovenous glucose difference/arterial glucose level: previously shown to be an adequate index of peripheral glucose utilization) were computed for each pair

of samples. In 23 experiments arterial glucose samples alone were obtained.

Comparison of the control (insulin) with glucagon (glucagon + insulin) periods revealed a significant ($P = 0.02$) increase in A-V/A values following glucagon administration in 11 (69 per cent) of the 16 patients; in the remaining patients no significant change occurred.

All experiments (39) were analyzed with regard to the relationship between glucagon/insulin ratio and arterial glucose levels. In 13 (mean ratio, 0.47) a neutralizing effect of the opposing action of the two agents on arterial glucose levels was obtained; in 24 (mean ratio, 0.76) hyperglycemia resulted; in 2 a biphasic reaction (ratio, 0.74) and blood sugar fall (ratio, 0.29) occurred.

This study indicates that a glucagon-insulin combination can produce greater enhancement of peripheral glucose utilization than insulin alone, as well as a stabilizing effect on arterial glucose levels. Such a combination, therefore, may prove useful in the management of some diabetic patients.

Starch Gel Electrophoresis of Serum Proteins and Urinary Proteins from Patients with Multiple Myeloma. RALPH L. ENGLE, JR., KENNETH R. WOODS, and JAMES H. PERT, New York, N. Y. (Introduced by Paul Reznikoff).

Because potato starch gel electrophoresis (Smithies) has high resolving power, it was used to study the serum protein and urinary protein patterns of 27 patients with multiple myeloma. Twenty urine samples containing Bence-Jones proteins and 14 sera were examined. Some urine samples were first concentrated by dialysis against polyvinylpyrrolidine. In other instances the urinary protein was precipitated by ⅔ saturation with ammonium sulfate, dialyzed, lyophilized, and applied to the gel as a 2 per cent solution.

Serum electrophoresis by this method gave more detailed and more distinct patterns than could be obtained by paper electrophoresis. The gamma-globulin was markedly decreased in all sera. Seven sera had single protein spikes in the gamma-globulin region; two sera had single spikes between the origin and slow-alpha$_2$-globulin. One serum showed marked elevation of slow-alpha$_2$-globulin; another, elevation of beta-globulin. Three sera deficient in gamma-globulin had no protein spike. One of these contained 3 gm. per cent macroglobulin (s = about 19S) which migrated as a gamma-globulin upon paper electrophoresis. With gel electrophoresis this macroglobulin did not migrate. All sera showed decreases in albumin and many demonstrated complex abnormalities in other components.

Analysis of the urinary proteins revealed that 6 samples contained 3 to 4 components, and 10 contained 2 components, all in high relative concentration, usually migrating close together. Four samples contained single components. This contrasted with the single components found in most instances by paper electrophoresis. Traces of albumin were frequently observed. One specimen of urinary protein for which no indication of heterogeneity could be obtained by paper electrophoresis, boundary electrophoresis at three hydrogen ion concentrations, ultracentrifugation, and immunologic studies by Dr. L. Korngold (Ouchterlony) was clearly shown by gel electrophoresis to contain two closely associated fractions (43 per cent and 52 per cent, respectively).

By means of starch gel electrophoresis new information has been obtained on the serum proteins in multiple myeloma and on the heterogeneity of Bence-Jones proteins.

The Effect of Exclusion of the Erythematous Palm Upon Venous Ammonia in Hepatic Cirrhosis. CURTIS J. FISHER, ROBERT EICH, and WILLIAM W. FALOON, Syracuse, N. Y. (Introduced by Eugene L. Lozner).

The association of palmar erythema with arteriovenous shunting of blood has been demonstrated in patients with hepatic cirrhosis. This observation may be of significance in studies of tissue metabolism as measured by arteriovenous differences. Arterial ammonia concentrations have been demonstrated to be appreciably greater than venous concentrations, suggesting the concept of peripheral utilization of ammonia, or as demonstrated by venous catheterization and *in vitro* studies that oxygenation may increase the "apparent" ammonia concentration. In an attempt to determine the effect of palmar erythema on venous ammonia, studies of arterial and venous ammonia and oxygen concentrations in 14 patients with liver disease, 9 with marked palmar erythema and 5 with minimal or no erythema, have been performed. In both groups of patients, studies were done before and during exclusion of the palm by a cuff inflated above systolic pressure.

Before exclusion no significant difference in the arteriovenous ammonia difference was observed between patients with erythema and those with none. Arteriovenous oxygen difference was less in patients with erythema. In contrast, with exclusion of the palm all patients with erythema showed a significant decrease in venous ammonia, averaging 45 per cent (range 27 to 75 per cent) of the control values. Thus a marked increase in arteriovenous difference in ammonia and oxygen occurred. Following removal of the cuff, these values returned to pre-exclusion levels. In patients with minimal or no erythema, exclusion had no effect upon venous ammonia or oxygen concentrations.

The results indicate that the presence of palmar erythema significantly alters the venous ammonia concentration. It is apparent that palmar erythema may similarly produce an error in measurement of other metabolites when peripheral arteriovenous techniques are used.

The Clearance Rate of I^{131} Labelled Streptokinase from the Circulation of Man. ANTHONY P. FLETCHER and ALAN J. JOHNSON,* St. Louis, Mo., and New York, N. Y.

The fibrinolytic state induced in man by the intravenous administration of streptokinase offers a possible approach to the treatment of thromboembolic disease.

Investigation of this phenomenon has been difficult in the absence of a method for the measurement of plasma streptokinase levels. Streptokinase of high purity and specific activity was labelled with I^{131} by the method of Hughes *et al.* There was no loss of activity during the labelling process and the integrity of the bond was maintained during electrophoresis.

The dose of streptokinase required to produce fibrinolysis in patients can be predicted by *in vitro* testing of the serum inhibitory factors. The dose so calculated is termed the predicted dose and a comparison of these values with the results of streptokinase infusion into man has shown that its accuracy is ± 15 per cent. Probit analysis showed that the predicted dose values in a sample of the population were distributed as a log normal variable.

Labelled streptokinase was cleared from the circulation at an exponential rate. The mean 50 per cent plasma clearance rate was extremely rapid, 18 min. (range, 5 to 35 min.) if a smaller dose than the predicted was infused, but slower, 81 min. (range, 58 to 110 min.) if the clearance determination was made after streptokinase equivalent to the predicted dose had been injected. These two clearance rates were significantly different ($p < 0.001$). Furthermore, there was evidence of rapid streptokinase breakdown as evinced by the early appearance of free I^{131} in the plasma and its excretion into the urine. Data are also to be presented on the levels of plasma streptokinase attained during continuous streptokinase infusion and on the accompanying changes in the components of the plasminogen-plasmin system.

The results indicate that the plasma streptokinase level is a crucial determinant in the fibrinolytic effect exhibited by plasma, after streptokinase infusion.

Systolic-Diastolic Pressure Relationships in the Isolated Beating Heart. Noble O. Fowler and Walter L. Bloom,* Atlanta, Ga.

Bloom and Ferris observed in the left ventricle of the excised submerged beating rat heart positive pressure during systole, and negative pressure during diastolic filling by suction.

In the present study, systolic-diastolic pressure relationships were observed in 33 excised beating rat hearts under three conditions: (1) spontaneous deterioration; (2) ventricular alternans; (3) during epinephrine or l-nor-epinephrine effect. Left intraventricular pressures were measured with reference to the hydrostatic pressure existing at the same level below the surface of the liquid in which the heart was submerged.

Results: Initially, peak systolic pressures were from 11 to 84 mm. water and negative diastolic pressures were from 2 to 24 mm. water.

In each of six hearts, regressions showed significant relation between peak systolic and diastolic nadir pressures during spontaneous weakening of the heart beat, $p < 0.001$.

With l-nor-epinephrine or epinephrine, peak systolic and negative diastolic nadir pressures increased. A regression of change in diastolic pressure upon change in systolic pressure was significant, $p < 0.01$.

Usually, ventricular alternans was associated with less negative diastolic pressure following each weak systolic beat. Thus strong systolic beats followed less negative diastolic pressures. One heart showed 25 mm. water systolic pressure and negative diastolic pressure of 8 mm. during strong beats, and systolic pressure of 5 mm. water with diastolic pressure of minus 1 mm. during alternate weak contractions.

The results indicated significant association between positive systolic and negative diastolic pressure in the excised beating heart under three conditions: (1) spontaneous decay; (2) increase in contractile force produced by sympathomimetic drugs; (3) ventricular alternans. It is suggested that diastolic force is related to systolic force. The results are consistent with the concept that the force of diastole is due to a release of energy stored in systole and may not be associated with a chemical release of energy.

Studies Using Glucose and Pentoses to Determine the Mechanism of the Hypoglycemic Response to Sulfonylureas. Thomas F. Frawley and Stanton Segal, Bethesda, Md. (Introduced by Richard T. Beebe).

Tolbutamide was given in amounts and by a route designed to produce a depression in blood sugar quantitatively similar to that obtained after insulin in the same individual. Following the rapid intravenous administration of sodium tolbutamide (18 mg. to 40 mg. per Kg.) or insulin (0.1 unit per Kg.) in two to three minutes an approximately 50 per cent decrease in blood glucose concentration was seen in thirty minutes. As with insulin the onset of the hypoglycemia after tolbutamide was immediate and accompanied by a depression in plasma phosphate. However, the tolbutamide-induced hypoglycemia was unaccompanied by the rise in blood pyruvate which occurs after insulin. When intravenous tolbutamide was substituted for insulin in the insulin-glucose tolerance test the hyperglycemic response following the hypoglycemia was suppressed, but not abolished, suggesting impairment of hepatic glycogenolysis. The glucose disappearance rate constant from blood after an acutely administered intravenous load of glucose (0.3 gm. per Kg.) was increased after intravenous tolbutamide or insulin. In one of the individuals studied a normal disappearance rate constant of 1.8 per cent per min. was increased to 4.0 per cent per min. with tolbutamide and to 7.2 per cent per min. with insulin, thus indicating that the tolbutamide effect on glucose disappearance closely parallels that of insulin. Twenty-gram quantities of D-xylose and L-arabinose were infused rapidly and the rate of disappearance from blood was compared after intravenous insulin and intravenous tolbutamide. Insulin accelerated the disappearance rate of both pentoses, decreasing the biological half-time to approximately one-third. Tolbutamide did not affect either pentose. The pentose observations do not suggest that tolbutamide enhances the release of and/or peripheral action of insulin. The com-

bined studies on glucose and pentose disposition suggest that tolbutamide's hypoglycemic effects are primarily hepatic. This might be brought about by altering hepatic responsiveness to endogenous insulin.

Metabolism of Albumin-Bound Labeled Fatty Acids in Man. DONALD S. FREDRICKSON and ROBERT S. GORDON, JR., Bethesda, Md. (Introduced by Robert P. Grant).

Previous studies indicate that the albumin-bound plasma unesterified fatty acids (UFA) may represent the major form in which fat is transported to tissues for oxidation. This hypothesis requires that plasma UFA turnover occur rapidly. The plasma turnover rate in man has been investigated by injecting intravenously C^{14}-carboxyl-labeled fatty acids bound to albumin and measuring the disappearance of radioactivity from plasma and its appearance in expired CO_2. Removal of radioactive UFA from the plasma followed a complex curve but followed first-order kinetics for 4 to 10 minutes after intravascular mixing. The half-time of disappearance during this phase varied from 1 to 3 minutes. Plasma turnover rates calculated by dividing the UFA concentration by the turnover time obtained from the early phase of the disappearance curve varied from 0.06 mEq. per liter per minute in subjects pre-fed carbohydrate to 0.5 mEq. per liter per minute in normals fasted 42 hours and in uncontrolled diabetics. Approximately 10 per cent of administered palmitate-1-C^{14} radioactivity was recovered as $C^{14}O_2$ in one hour in the fasted subjects, less in those fed carbohydrate. The peak specific activity occurred at 25 minutes. In two subjects, linoleate-1-C^{14} radioactivity appeared more slowly. The preliminary studies indicate that: 1) UFA molecules leave the plasma rapidly despite very tight binding to albumin; 2) the turnover of plasma UFA appears to be closely related to the contribution of fat to the total metabolic pool; 3) plasma UFA does not mix with a pool as great as the total depot fat prior to oxidation, although the origin of plasma UFA in fasting subjects appears to be the adipose tissue.

Clinical Application of Lupus Serum—Nucleoprotein Reaction Using the Fluorescent Antibody Technique. GEORGE J. FRIOU, West Haven, Conn. (Introduced by S. C. Finch).

Use of the fluorescent antibody technique has revealed that a factor in lupus serum adheres to nuclei in sections of animal tissues. More recently, this observation has been extended to normal human tissues.

Nucleohistone (TNH) was prepared from calf thymus and tested for participation in this reaction. It was found that sera which caused localization of fluorescent anti-human globulin on nuclei also caused it to adhere to spots of TNH on glass slides. Sera not causing nuclear localization were also negative with TNH. Additional evidence of the identity of the reactions was obtained by parallel tests against nuclei and TNH spots after absorption of positive sera with precipitated TNH.

Reproducible titers could be obtained by testing dilutions of serum against TNH spots. A large number of sera from patients with lupus and other diseases were tested by this method. Results indicate presence of the factor to be characteristic of lupus. Reactions at low dilution have also been observed with several sera from among the groups of patients with rheumatoid arthritis and allergic reactions to drugs, but not in other diseases.

Incomplete information indicates the probable identity of the serum factor with the factor responsible for L.E. cell formation. Because of the sensitivity of this method, and the semi-quantitative results that can be obtained, it should be applicable to study of many unanswered questions regarding lupus.

Effect of Acetylcholine in the Pulmonary Circulation of Man. H. W. FRITTS, JR., P. HARRIS, R. H. CLAUSS, and A. COURNAND, New York, N. Y. (Introduced by D. W. Richards).

One of us (P. H.) has observed that a single injection of acetylcholine can lower the pulmonary arterial pressure in patients with a moderate degree of pulmonary hypertension. Since the effect was not evident in normal subjects, it was suggested that the response to the drug depends on the pre-existing vascular tone. This hypothesis was based on two considerations: 1) the fact that in patients with pulmonary hypertension the muscular coats of the small vessels are hypertrophied, and 2) the assumption that this hypertrophy and an increased tone go hand in hand. In the present study, the hypothesis has been tested by using hypoxia to raise the tone of the pulmonary vessels of 8 normal subjects. Acetylcholine was infused into the main pulmonary artery at the rate of 0.5 mgm. per min. While the subjects breathed 21 per cent oxygen, the drug consistently lowered the systolic pressure in the pulmonary artery by from 1 to 7 mm. Hg. The mean pressure was also lowered, and the diastolic pressure inconstantly reduced. No change was apparent in the pulmonary wedge pressure, the systemic blood pressure, the heart rate, or the cardiac output. After hypoxia had produced pulmonary hypertension, the fall in pressure with acetylcholine was more pronounced, despite the fact that during the infusion the cardiac output frequently increased. The mechanism of the effect is not known, although the same response was observed in one patient who had had all of the sympathetic ganglia removed. These results bear on the problem of vasomotricity in the lungs, and suggest that under certain conditions the pulmonary vessels have the capacity for intrinsic changes in tone.

Mechanism of the Glucosuria Produced by the Administration of Steroids with Glucocorticoid Activity. E. RUDOLF FROESCH, ALBERT I. WINEGRAD, and ALBERT E. RENOLD, Boston, Mass. (Introduced by George W. Thorn).

The mechanism of the glucosuria produced by adrenal corticoids has been investigated in four healthy young males and in two patients with renal glucosuria. *Each subject served as his own control.* In the normal subjects glucose titration and renal clearance studies were

carried out on control days as well as 12 and 90 hours after instituting prednisone administration by mouth. Blood and urine glucose were measured by a specific enzymatic method and inulin determinations were carried out after enzymatic removal of interfering glucose. Each study consisted of at least ten consecutive periods. In all subjects prednisone administration was associated with increased blood glucose levels and an increased rate of glomerular filtration (inulin). These two factors accounted for the increased glucosuria. In no instance was TmG affected significantly nor was increased glucose excretion noted at glucose loads below TmG. The two patients with renal glucosuria were kept fasting for 16 hours prior to hydrocortisone administration and throughout the study. Hydrocortisone was administered intravenously over ten hours. Blood glucose increased markedly in both subjects and urinary glucose excretion increased from 10 to 36 mg. per minute and from 5 to 25 mg. per minute, respectively. Again the increase in blood glucose and the increased glomerular filtration rate accounted for the increased urinary glucose excretion. Tubular glucose reabsorption was not significantly altered. There was a lag of 3 to 4 hours between the beginning of intravenous hydrocortisone administration and the onset of either the blood glucose or the glomerular filtration rate response. In two patients with Cushing's Syndrome and glucosuria TmG was measured and found to be within normal limits. It is concluded from these studies that the glucosuria produced by the administration of certain adrenal corticoids results from an increase in the filtered glucose load, not from decreased tubular glucose reabsorption.

Relation of Acquired Hemolytic Anemia to Macroglobulins. HUGH FUDENBERG and HENRY G. KUNKEL,* New York, N. Y.

The sera of five patients with the syndrome of high titer cold agglutinins, paroxysmal cold hemoglobinuria, and chronic hemolytic anemia were studied by (starch) zone electrophoresis and analytical and (density gradient) zone ultracentrifugation. Titers ranged from 1,000 to 60,000 at the time of study. The two sera with the highest titers showed abnormal peaks at different points in the β-γ-globulin region upon zone electrophoresis. The component responsible for the peak in each instance was isolated; titrations in parallel disclosed that cold agglutinin activity was confined almost exclusively to this peak. Ultracentrifugation studies of the isolated components proved them to consist primarily of high molecular weight material with a sedimentation coefficient of 19S. Zone ultracentrifugation studies of the isolated components proved them to consist primarily of 19S material. Zone ultracentrifugation confirmed the 19S character of the cold agglutinin.

The three sera with lower titers failed to show an abnormal component by electrophoresis; however, the cold agglutinins were found to sediment as 19S proteins in the density gradient system.

One case of paroxysmal cold hemoglobinuria of the Donath-Landsteiner variety was also studied and showed "antibodies" of 19S character. This was confirmed through the use of specific antiserum to 19S γ-globulin.

In contrast, the "antibody" activity of the sera of 2 patients with acquired hemolytic anemia of the warm variety, with positive direct and indirect Coombs tests, was due to γ-globulins of 7S character rather than to 19S macroglobulins. Direct Coombs reactions were obtained with the use of anti-7S human γ-globulin rabbit serum while no visible reactions occurred with anti-19S γ-globulin rabbit serum. Zone ultracentrifugation of the sera in these cases also showed the 7S nature of the "antibody" protein.

Studies on the Ground Substance in Myxedema. II. Effect of Desiccated Thyroid, L-Triiodothyronine and Cortisone in Myxedema, Localized Myxedema, and Severe Exophthalmos. J. LESTER GABRILOVE, New York, N. Y. (Introduced by Louis J. Soffer).

Without anesthesia skin was obtained at frequent intervals from the same area by punch biopsy and studied histologically and histochemically. The metachromatic material present in the skin of myxedematous patients disappeared during the course of treatment with desiccated thyroid and reappeared when specific therapy was withdrawn. The administration of l-triiodothyronine caused a more rapid disappearance of the metachromatic material than was noted with the use of desiccated thyroid. Similarly when l-triiodothyronine was withdrawn, the histochemical appearance characteristic of myxedema promptly recurred. The amount of metachromasia in the myxedematous skin was also decreased by the systemic administration of cortisone alone.

The simultaneous occurrence of localized (pretibial) myxedema and malignant exophthalmos is common. It has been reported from this laboratory that in experimental exophthalmos there is an increased deposition of mucoprotein. The injection of hyaluronidase into an area of pretibial myxedema or generalized myxedema rapidly resulted in pitting of the lesion and a decrease in the amount of metachromatic material demonstrable on histologic examination. A single injection of cortisone or of l-triiodothyronine into the region involved in pretibial myxedema effected a decrease in the quantity of the mucinous material in the lesion over a period of several days. The systemic administration of cortisone also altered the lesion in similar fashion.

In patients with malignant exophthalmos of short duration, the edema of the lids was made to pit by the injection of hyaluronidase and caused to decrease by the systemic administration of cortisone. Biopsy of these lesions was not feasible.

The significance of these observations is discussed.

Treatment of Abnormal Regulation of Respiration in Pulmonary Emphysema by Pharmacologic Means. MORTON GALDSTON,* New York, N. Y.

Advanced emphysema patients exhibit a diminished ventilatory response to CO_2 and respiratory depression

upon sudden relief of anoxemia by inhalation of oxygen which sometimes results in CO_2 narcosis. Treatment of this complication with a mechanical respirator is often unsatisfactory; therefore, other means were investigated.

Studies with either 20 cc. intravenous Aminophylline (0.5 G) or 0.85 N saline and oral Diamox® (250 mgm. every 12 hours) alone and together during inhalation of mixtures of approximately 3 and 5 per cent CO_2 in O_2 and also 100 per cent O_2 in two patients indicate that these abnormal ventilatory responses can be altered. Aminophylline administered after the metabolic effects of Diamox® had stabilized nearly doubled minute ventilation as compared with saline-control, when measured at the same level of arterial pCO_2 attained with the CO_2-O_2 mixtures in the different periods. Little or no ventilatory stimulation occurred with Aminophylline or Diamox® alone. However, in an individual with a normal respiratory regulatory mechanism Aminophylline, Diamox® and both together respectively caused 2-, 3- and 4-fold ventilatory increases.

Aminophylline, but not saline or Diamox®, promptly overcame respiratory depression during oxygen inhalation and sustained minute ventilation 15 to 25 per cent above room air levels over the next ¾ of an hour of observation. Arterial pCO_2 was then 7 to 11 mm. Hg below and pH 0.06 units above oxygen-saline period levels, when arterial pCO_2 rose 3 to 10 mm. Hg and pH fell 0.03 to 0.06 unit as compared with room air. When Aminophylline was administered with Diamox®, again arterial pCO_2 fell 11 to 13 mm. Hg while pH remained unchanged or fell only 0.03 unit. Aminophylline-stimulated respiration was still present during intermittent oxygen inhalation continued for two and one-half hours.

Plasma Concentrations of Epinephrine and Norepinephrine in Normal Individuals, with Observations on the Arteriovenous Difference and In Vitro Disappearance of the Amines. Hugo Garcia and John Wallace, Durham, N. C. (Introduced by Eugene A. Stead, Jr.).

Mental and physical activities are known to affect blood levels and rate of urinary excretion of epinephrine and norepinephrine. Subjects at rest might be expected to have a different range of normal values from subjects carrying on usual activities. Two groups of normal persons were studied: 15 subjects after 45 to 60 minutes of complete bed rest and 15 subjects during regular work. Arteriovenous differences of epinephrine and norepinephrine in a group of 30 normal individuals were also examined.

Samples were assayed fluorometrically and in duplicate, using a simplified and standardized technique based on the Aronow and Howard modification of the ethylenediamine method. Final technique was standardized after a re-evaluation of all steps involved in the original procedure and re-examination of previously raised criticisms concerning the methodology. Sensitivity permits quantitative measurement of 0.25 μg. epinephrine per liter plasma and 0.5 μg. norepinephrine per liter plasma differences. Adding mixtures of both amines to plasma samples, 93 ± 13 per cent and 84 ± 15 per cent, respectively, are recovered. Method was tested in detecting amines after parenteral administration to humans and to demonstrate slow disappearance of epinephrine and norepinephrine *in vitro*.

Epinephrine was found present in only 2 of the 15 individuals at rest, average norepinephrine levels for the group being 2.2 ± 0.54 μg. per L. The ambulatory group showed detectable amounts of epinephrine (average 0.30 ± 0.21 μg. per L.) in 14 of the 15 subjects, with average norepinephrine concentration of 3.2 ± 0.94 μg. per L. Differences are statistically significant ($P < 0.01$). The values differ from ones reported originally by Weil-Malherbe and Bone.

The venous blood had a higher norepinephrine concentration. In 30 subjects, average arteriovenous difference was minus 0.36 μg. per L., $P < 0.01$. Epinephrine levels, when detectable, showed random distribution in arterial and venous samples.

Data show: no detectable epinephrine at rest; increase of both catechols on activity; negative arteriovenous norepinephrine difference.

A New Concept in Prosthetic Material for Use in Plastic Cardiac Surgery. Goffredo G. Gensini, Vicente N. Roger, Robert L. Hawley, and Gardner Middlebrook,* Denver, Colo.

No ideal prosthetic substance for plastic cardiovascular surgery has yet been found. A material specially prepared from sheep caecum and used for the manufacture of condoms was selected for investigation. This paper-thin laminate membrane was chosen because of its mammalian origin, pliability and strength.

This investigation was designed to determine the extent to which this substance could be functionally incorporated into vascular tissues. The experimental animals used were dogs and this material was grafted into arteries, veins and heart. Artificial semilunar valves were constructed in aortas.

At first, leukocytes and fibrin appeared in the spaces between the laminae. Then fibroblasts rapidly migrated from the host tissue through the spaces, utilizing the laminae as a framework. Within two months replacement by host tissue was complete. This prosthetic material elicited no significant foreign body reaction. The artificial semilunar valves retained their original shape with free edges, open sinuses and appeared to have increased strength. The leaflets were slightly but uniformly thickened and somewhat smaller. Thrombotic phenomena did not occur on the heart grafts or on the valves. However, the pulmonary artery grafts became thrombosed.

The rate of fibrosis of the prosthesis appears to be adequate to insure retention of the graft. This is probably due to guiding of the proliferating fibroblasts through the interstices of the laminae, resulting in a laminated structure with great strength.

The concept of a cardiovascular prosthesis being completely replaced by multi-layered fibrous connective tissue

of the host appears to represent an advance over previously used irreplaceable, nonlaminated substances. The material described seems, in particular, to possess superior qualities for the construction of artificial cardiac valves.

The Anabolic Significance of the Fecal Dipyrrylmethene Pigments or Mesobilifuscin Group. A. SIGRID GILBERTSEN and P. T. LOWRY, Minneapolis, Minn. (Introduced by C. J. Watson).

The compound which Siedel designated as mesobilifuscin (Mbf) is found in the feces partly as a dark brown pigment, partly as a colorless chromogen readily converted to the pigment. Infra-red spectroscopic study of this and of the fecal pigment earlier designated as copronigrin has revealed close, if not complete, identity. Siedel's analysis of the fecal Mbf and of that obtained by appropriate treatment of mesobilirubinogen, *in vitro*, indicates a dipyrrylmethene structure, in contrast to the tetrapyrrylmethene chain of the bilirubinoids. It has been generally assumed that the fecal dipyrrylmethene is derived by oxidative schism of bilirubinoid pigments. In the present study, however, the labelling of N^{15} mesobilifuscin following administration of N^{15} glycine, unlike that of stercobilin, is largely limited to the early period, being small or relatively insignificant in comparison with that of stercobilin, at the time of destruction of mature circulating erythrocytes. This indicates that the compound is mainly anabolic rather than catabolic in origin and that it may be derived from unused pigment in the sense of a pigment "complex" or pool, such as first suggested by Whipple in 1922. Further indication of anabolic character is provided in the comparison of amounts of fecal urobilinogen and Mbf in cases of refractory or aplastic as contrasted with hemolytic anemia. In the former the values are much below normal, even when the urobilinogen is normal; in other words, the U/Mbf ratio is characteristically large. In hemolytic anemia the amounts are large and the U/Mbf ratio is normal or reduced.

In ten cases of liver disease thus far studied the fecal Mbf has been remarkably low. The possible significance of this will be discussed.

Cellular Damage Associated with the Delayed Hypersensitive State in Mumps Virus Infection. LOWELL A. GLASGOW and HERBERT R. MORGAN,* Rochester, N. Y.

A state of delayed hypersensitivity to mumps virus develops during the disease in man and a similar state has been produced in guinea pigs infected with mumps virus. In either host, this hypersensitive state is manifested *in vivo* by a delayed type of dermal reaction to the injection of heat-inactivated virus. In the guinea pig it can be shown that dermal sensitivity is associated with a cellular hypersensitivity by studies *in vitro*. Explants of splenic tissue from such animals are cultivated on a plasma coagulum in a medium consisting of guinea pig serum, ox serum ultrafiltrate, and a balanced salt solution. From such explants, macrophages migrate into the surrounding plasma coagulum. With the addition of mumps viral antigen there is a marked inhibition of the migration of macrophages from the splenic tissue, and after continuing contact with the antigen these cells round up, become vacuolated, and finally undergo lysis. Similar exposure of splenic tissue from normal guinea pigs to mumps viral antigen is without effect. This cytotoxic action of the mumps viral antigen can be abolished by addition of cortisone to the culture medium prior to the introduction of the viral antigen.

The implications of these findings in the natural disease in man, in which the hypersensitive state can be shown to be present during the development of symptoms, will be discussed from the standpoint of the possible role of this state in the symptomatic manifestations of the disease through the cellular damage produced by the interaction of viral antigen and the hypersensitive cell.

A Comparison of Certain Aspects of Calcium Kinetics in Hyperparathyroidism and Paget's Disease of Bone. RICHARD GOLDSMITH, Cincinnati, O. (Introduced by Morton Hamburger).

Hyperparathyroidism and Paget's disease of bone are believed to be associated with increased formation and destruction of bone. The present investigation was initiated to provide a better understanding of the abnormal physiology of these two diseases and the differences between them. Calcium studies utilizing balance and radioactive tracer techniques were conducted on 3 patients with active primary hyperparathyroidism (one had X-ray bone disease) and on 2 patients with active, widespread Paget's disease. Each patient was in negative calcium balance when studied. The determinations of stable and radioactive calcium content were performed on serum, urine and stool. The apparent size of the available calcium pool in each patient with Paget's disease was more than ten times greater than in each subject with hyperparathyroidism. The size difference was obvious within a few hours after IV administration of radiocalcium and increased with time. The total excretion of radiocalcium by the patient with Paget's disease studied for the longest time interval was 4.3 per cent of the administered dose; the excretion in the patient with hyperparathyroidism studied for a similar interval was 20.6 per cent of the administered dose.

One patient with Paget's disease was given 500 milligrams of cortisone daily to see what effect this antianabolic agent would have on calcium metabolism. The degree of negativity of the calcium balance was considerably reduced and the excretion of labeled calcium ceased. A hyperparathyroid subject similarly studied with 300 milligrams of cortisone daily showed no change. The most likely explanation for all of these findings is that in Paget's disease there is a much slower movement of calcium between bone forming and bone resorbing surfaces than in hyperparathyroidism. This is true in the patients studied even though bone resorption in both was equally enhanced as evidenced by equivalent degrees of negative calcium balance. These data also suggest that the mechanism of the bone resorption in the two diseases

is different. Finally, it seems most consistent with the data to assume that cortisone benefits the patient with Paget's by slowing bone breakdown.

Delayed Allergy and Agammaglobulinemia. ROBERT A. GOOD,* SOLOMON J. ZAK, DONNA R. JENSEN, and A. M. PAPPENHEIMER, JR., Minneapolis, Minn.

Much interest concerns the nature of delayed allergy. Particularly incisive in the study of relationships of bacterial allergy to other immunologic mechanisms are agammaglobulinemic patients. Chosen for this study were six male children with congenital and three adults with acquired agammaglobulinemia. In spite of prior observations indicating that these patients lack delayed allergy to pneumococcal and streptococcal antigens, the present studies indicate, in agreement with Porter, that agammaglobulinemic patients can develop bacterial allergy.

1. Bacterial allergy to streptococcal products and tuberculin is transferred with cells to agammaglobulinemic patients in whom it persists for two years, indicating that these patients can sustain bacterial allergy.

2. Delayed allergy to 2,4-dinitrofluorobenzene is regularly produced by its application to skin of either normal or agammaglobulinemic patients. This form of allergy is regularly transferred from sensitized normal or agammaglobulinemic patients to non-sensitized recipients with large numbers of viable circulating leukocytes.

3. Normal and agammaglobulinemic adults and children are regularly sensitized to diphtheria toxoid by the intradermal injection of specific diphtheria toxoid—horse antitoxin precipitates. No sensitivity to horse antitoxin is produced.

4. Conversely, agammaglobulinemic patients and normal persons develop dermal sensitivity to horse gamma globulin when the latter is injected as specific precipitate with anti-horse gamma globulin rabbit serum. No sensitivity to rabbit serum is produced.

The delayed allergy developed by these means may be transferred from sensitized normal or agammaglobulinemic patients to normal persons by injection of viable leukocytes from members of either group.

These observations indicate that the mechanism for development of delayed allergy is intact in agammaglobulinemic patients and tend to dissociate bacterial allergy from classical antibody production. Their implication to immunological theory and their relationship to the occurrence of fibrinoid disease and arthritis in agammaglobulinemic patients will be considered.

Further Studies of the Use of the Ear Oximeter for Repeated Measurement of Cardiac Output at Rest and During Exercise. ALLAN V. N. GOODYER* and ALLEN CHETRICK, New Haven, Conn.

The determination of cardiac output by means of dye dilution curves recorded by ear-densitometry has required calibration of each curve either by one or more arterial samples, or by a 5-minute venous sample. The arterial cannulation adds difficulty and discomfort to the method, and the use of the 5-minute sample involves not only a large extrapolation from a non-linear region of the cuvette response curve, but also a degree of stability of the oximeter baseline which has, in our experience, proven very difficult to obtain, particularly during exercise.

The present study explored the use of a standard calibration factor for one earpiece, based on the means of the peak values of 22 simultaneous oximetric and arterial dye curves in 12 subjects. The correlation between paired peak values was found to be quite good ($r = +0.81$, S.E. $= 0.21$). If only repeated determinations in the same subject are considered, the correlation of simultaneous peak values was even better ($r = +0.92$, S.E. $= 0.33$).

Simultaneous determinations of cardiac output with the above method and by the Fick procedure, both at rest and during exercise, were closely comparable ($r = +.91$, S.E. $= 0.28$). Indigo Carmine (as reported by Newman), but not Methylene Blue, was found to be a good substitute for T1824. The mean error of the dye method was -4 per cent ± 16 per cent, as compared to the Fick method.

The ear oximeter, therefore, used with a standard calibration factor, and with certain other precautions, would appear to be applicable to the repeated measurement of large changes of cardiac output during exercise in human subjects, particularly where relative values in the same subject are concerned.

The Effect of Acetazolamide (Diamox®) on Calcium and Citrate Metabolism in Humans. EDWIN E. GORDON and SHELDON G. SHEPS, Boston, Mass. (Introduced by A. S. Freedberg).

The physico-chemical properties of calcium and citrate in aqueous solution, and in blood, have been investigated intensively. It has been postulated that the citrate present in urine binds calcium with the formation of a soluble and poorly dissociable complex, thus preventing the precipitation of insoluble calcium salts. Harrison and Harrison have recently provided experimental evidence to support this view, demonstrating a marked reduction in urinary citrate excretion with precipitation of calcium salts in the urinary tract of rats which were fed acetazolamide (Diamox®).

We have studied the citrate and calcium concentrations in the blood and urine of a patient who developed urolithiasis while receiving Diamox®. In addition, citrate and calcium metabolism was studied in five normal subjects before, during and after Diamox® administration.

Diamox® (250 mgm. t.i.d. for three days) reduced the urinary citrate excretion in all the subjects. In two subjects, citrate excretion was reduced to 10 per cent of control levels; in the remaining four subjects a fall to 25 to 33 per cent of control values was observed. Statistical evaluation of these data showed a z value which falls beyond the 0.001 level of significance. Serum citrate and calcium concentrations and urinary calcium content were not altered. Diamox® administration thus increased the calculated ionized calcium in the urine (potentially precipitable calcium) from an average

value of 51 per cent to an average value of 93 per cent of total urinary calcium. This effect of Diamox® was apparent six hours after the administration of a single dose of the drug.

Further studies to clarify the mode and site of action, the dose relationships and the duration of effect of Diamox® on citrate and calcium metabolism are currently in progress.

The Effect of Nitroglycerin on the Coronary Circulation and Myocardial Metabolism in Man. RICHARD GORLIN,* JOHN M. BOZER, and JOHN W. VESTER, Boston, Mass., Portsmouth, Va., and Pittsburgh, Pa.

The capacity of the coronary vascular bed to dilate following administration of nitroglycerin has been studied in patients with mild and severe heart disease of varied etiology. Following coronary sinus catheterization the nitrous oxide technique was utilized to measure coronary blood flow in 12 patients with normal left ventricular (LV) work and minimal symptoms, and in 3 patients with greatly increased LV work. Nearly simultaneous estimations of coronary flow, myocardial oxygen consumption, cardiac output (Fick or indicator dilution method), systemic arterial pressure, and heart rate were made before and 10 minutes after 0.6 mg. nitroglycerin sublingually.

A linear correlation ($R = 0.81$, $p = .01$) has been found between myocardial oxygen consumption per second of systole and resting LV work in Kg. M per min. per M^2. Oxygen consumption increased not only with increased work but also (for the same work) directly with heart rate and the number of seconds spent in contraction per minute. In the nearly normal patients, following nitroglycerin, both coronary flow and myocardial oxygen consumption increased (57 per cent) while coronary vascular resistance (CVR) decreased 50 per cent. Increase in oxygen consumption was out of proportion to minor changes in work or heart rate. Calculated cardiac efficiency decreased. In the patients with increased LV work, CVR and therefore coronary flow and myocardial oxygen consumption did not change significantly; cardiac work decreased disproportionately and efficiency declined. The fixed coronary resistance and flow in the severe cardiacs may indicate that maximal coronary vasodilatation had already been reached in response to the greatly increased work loads and oxidative energy demands.

These studies would suggest that nitroglycerin altered myocardial energy metabolism and that either coronary flow increased secondarily or that cardiac work decreased when coronary flow could not be increased.

Psychological Factors and the Manifest Development of Leukemia and Lymphoma in Humans. WILLIAM A. GREENE, JR., Rochester, N. Y. (Introduced by George L. Engel).

Proponents of any one factor, virus, chemical, radiation, or hormone as an initiating etiological agent in leukemia or lymphoma consider the probability that other factors may play a precipitating role, perhaps through modification of host resistance.

Study of 100 patients has shown that the disease developed in a setting of separation with depression in 93 of 100 patients. Separation is considered to be a psychophysiologic state occurring in situations of loss of a significant person or goal with an accompanying affect of conscious or unconscious sadness or hopelessness and usually associated change in motor, gastrointestinal, and vascular activity.

Unselected patients showed the following kinds and frequency of unresolved separation occurring at a median point of 12 months before apparent onset in the adults and 6 months before apparent onset in the children.

(1) Men: 22 patients; leukemia 12, lymphoma 10. Separations for the men included loss of a significant person, mother, wife, father, advent of an offspring, a daughter leaving home, change of job, or retirement. Such separations had occurred in 20 of the 22 men.

(2) Women: 40 patients; leukemia 22, lymphoma 18. Separations for the women included loss of a significant person, mother, husband, father, child, the menopause and change of home. Such separations had occurred in 38 of the 40 women.

(3) Children: 38 patients; leukemia 33, lymphoma 5. Separations for the children included birth of a sibling rival, change of home, change of school, loss of father, loss of a substitute mother. Such separations had occurred in 35 of the 38 children.

These data indicate that many of the prodromal symptoms are related to psychological processes as well as to the developing lymphoma or leukemia. Further, it is considered that significant separation may be a frequent condition precipitating the manifest development of leukemia and lymphoma.

In Vivo Capillary Alterations in Man During Hemorrhagic Fever and Other Infectious Illnesses. SHELDON E. GREISMAN, Baltimore, Md. (Introduced by Charles L. Wisseman, Jr.).

Clinical and pathologic evidence points to a significant degree of small vessel dysfunction in certain infectious diseases such as epidemic hemorrhagic fever and some rickettsial infections which *per se* might explain important features of these diseases. In order to study the dynamic state of the small vessels and to correlate this with the clinical and pathologic picture, serial direct microscopic observations of the nailfold capillaries during the course of illness were performed in 71 patients with hemorrhagic fever and 51 patients with various other infectious illnesses. The nailfold capillary bed, studied under standardized conditions, provided a segment of the minute vascular system anatomically and functionally comparable in all patients. Although individual variations were marked, certain patterns emerged:

1) Hemorrhagic fever: Decreased vasomotor activity, refractoriness to l-nor-epinephrine, increased mean capillary diameter, and hemorrhagic diathesis characterized the febrile and hypotensive phases. Slowing of blood

flow and sludging were less conspicuous. During the hypertensive and diuretic phases, capillary constriction and heightened vasomotor activity developed, gradually reverting to normal during convalescence.

2) Other infectious illnesses: Most other patients, despite pyrexia to 105° F, exhibited no significant nailfold capillary alterations. However, two subjects with clinical evidence of generalized and severe capillary injury during scrub typhus and Rocky Mountain spotted fever, and one patient with infectious mononucleosis, manifested serial capillary changes indistinguishable from those noted during the hypotensive phase of hemorrhagic fever. Nine patients with early Q fever failed to exhibit any significant nailfold capillary changes, conforming with the concept that *R. burneti* lesions are limited to the pulmonary area in man.

The findings indicate that the nailfold capillary reaction to a variety of infectious agents follows a definite pattern which can be correlated with the clinical and pathologic features of the illness.

Effects of Corticotropin, Hydrocortisone, and Stress on Albumin Synthesis and Catabolism in Man. JACOB GROSSMAN, A. AARON YALOW, and RAYMOND E. WESTON,* New York, N. Y.

The effects of stress and/or adrenal corticoids on albumin, in contrast to general protein, metabolism have received little attention. Because the use of I^{131}-labelled human serum albumin (RISA) permits, in the absence of severe disequilibration, independent estimation of albumin breakdown and synthesis, the presumed anti-anabolic, as well as catabolic, effects of adrenal cortical hormones on albumin may be studied. The catabolic rate is the daily percentage decrease of retained radioactivity, measured from urinary I^{131} excretion, while anabolism is reflected by the percentage rate of decrease in specific activity of plasma albumin.

Sixteen to eighteen days following intravenous injection of RISA, to permit equilibration plus adequate control periods, corticotropin (80 to 120 U daily) or hydrocortisone (200 to 300 mg. daily) was administered for 6 to 9 days to nine subjects (normal or cardiac) under metabolic ward control, including fixed dietary and fluid intake and moderate activity. Balances of electrolytes and nitrogen and daily urinary excretions of creatinine and 17-hydroxycorticoids were measured.

Control albumin turnover varied from 0.023 to 0.057 per day; synthesis approximated breakdown. Hormone administration was associated with prompt, sustained increase in urinary I^{131} excretion to almost twice the control rate, reflecting increased albumin breakdown, followed by gradual subsidence despite continued therapy. Anabolic effects were delayed several days. Albumin synthesis increased on corticotropin, except in one subject, but was unchanged or slightly decreased on hydrocortisone. In two patients who developed spontaneous infections, markedly increased breakdown and severely diminished synthesis of albumin occurred.

The apparent contribution of the increased albumin catabolism to the negative nitrogen balance was unusually large relative to the ratio of exchangeable albumin to total body protein. The implications of this observation will be discussed.

Demonstration of Erythropoietin in Human Plasma. CLIFFORD W. GURNEY, EUGENE GOLDWASSER, LEON O. JACOBSON,* and CHAO PAN, Chicago, Ill.

Demonstration of plasma erythropoietin activity has been facilitated by the finding that hypophysectomized or acutely starved rats give an exaggerated response to fractionated animal or human anemic plasma. It has been demonstrated repeatedly that the increased incorporation of Fe^{59} in rat erythrocytes is a reliable index of erythropoietic activity induced by injections of such fractionated plasma. Two 2-ml. injections of heat-denatured or ethanol-extracted normal human plasma failed to increase the per cent of Fe^{59} incorporated into the erythrocytes 16 hrs. after injection of a tracer dose of $Fe^{59}Cl_3$. However, ethanol-extracted normal human plasma, concentrated 30 times, enhanced erythropoietic activity, increasing Fe^{59} incorporation two fold. Unconcentrated extracts of heat-denatured plasma from a patient with hypoplastic anemia consistently produced a three- to five-fold increase in Fe^{59} uptake in hypophysectomized or starved rats. After transfusion raised the hemoglobin level, plasma activity could no longer be demonstrated. Activity was demonstrated in plasma from patients with pernicious anemia, blood loss anemia, aleukemic leukemia, Hodgkin's disease, acute hemolytic anemia, and hypoplastic anemia. It was not found in plasma from anemic patients with uremia, advanced neoplasms, or malnutrition. If the anemia is mild, concentration of the extract often permits demonstration of activity. Increased erythropoietin concentration has also been demonstrated in the non-dialyzable fraction of urine from some anemic patients. We conclude that erythropoietin is present in normal plasma and that its concentration is increased in plasma from patients with a variety of anemias. Our data are consistent with the concept that erythropoietin is a hormone that regulates the rate of erythropoiesis. The anemic state usually induces a compensatory increase in this factor. Suboptimal levels or failure to respond to anemia by increasing the plasma erythropoietin concentration is postulated to be of importance in the pathogenesis of some anemias.

Peripheral Arteriovenous Shunting Mechanisms in Patients with "S" Type Hemoglobin. E. RICHARD HALDEN, BRIAN J. SPROULE, and E. E. MUIRHEAD,* Dallas, Tex.

During investigation of the *in vivo* pO_2-sickling relationship, marked variation in per cent sickling occurred in different venous blood samples from the same subject. Possible causes of the phenomenon were studied in 18 subjects possessing S-type hemoglobin.

Arterialization of venous blood was demonstrated in 15 subjects by analyzing simultaneously drawn peripheral venous, arterial and central venous (7 subjects) blood for

pO_2 (polarograph), oxygen saturation and degree of sickling. Pure oxygen breathing was used to render peripheral arteriovenous admixture more apparent.

Nine individuals showed increased oxygenation of venous blood while breathing room air ($\overline{SvO_2}84$, $\overline{PvO_2}55$, $\overline{SaO_2}91$, $\overline{PaO_2}68$). Oxygen breathing caused marked increase in saturation of venous blood and in PvO_2 (175 mm. Hg) in five. In the remaining four no increase occurred.

Six other subjects showed no evidence of peripheral arteriovenous admixture during ambient air breathing ($\overline{SvO_2}60$, $\overline{PvO_2}32$, $\overline{SaO_2}93$, $\overline{PaO_2}74$) but did show such admixture during pure oxygen breathing ($\overline{SvO_2}89$, $\overline{PvO_2}69$).

Arterialization was most often demonstrated in veins of the forearm and was influenced by the concentration of inspired oxygen. Inhalation of pure oxygen by 5 subjects resulted in a fall in peripheral SvO_2 (mean decrease 16 per cent) but caused an increased saturation of simultaneously drawn pulmonary artery blood.

Twice during low oxygen and once during room air breathing SvO_2 was higher than SaO_2. The degree of sickling was correspondingly less in the venous than in arterial blood, suggesting that segregation of desaturated sickled cells from more oxygenated discoid cells occurred in A-V communications.

Individuals possessing S-type hemoglobin appear to have functional peripheral A-V communications which, along with the established relationship of sickling to pO_2, offer a possible explanation of discrepancies in peripheral sickling in such patients.

Studies on the Spinal Fluid and Serum Protein Electrophoretic Pattern in Patients with Diabetes Mellitus. WILLIAM J. HAMMACK, WILLARD R. STARNES, and S. RICHARDSON HILL, JR., Birmingham, Ala. (Introduced by Ben Friedman).

Because of the elevated spinal fluid protein levels and abnormal serum protein patterns present in some patients with degenerative complications of diabetes mellitus, studies have been undertaken to characterize further the serum and spinal fluid protein patterns in forty such patients. Electrophoretic studies were simultaneously performed on serum and concentrated spinal fluid. The results have been expressed in per cent and milligrams. In diabetic patients without degenerative complications, the protein patterns of the serum and spinal fluid were normal. Patients with diabetic neuropathy had a normal serum protein pattern, but an elevated spinal fluid protein, consisting of increases in each fraction, especially in gamma globulin. This may indicate the presence of other mechanisms, in addition to a generalized increase in vascular permeability. Patients with diabetes mellitus and persistent proteinuria had a lowered spinal fluid total protein and albumin fraction, but an elevated alpha and beta globulin fraction. The serum was characterized by a decreased albumin and an increased alpha and gamma globulin. The decrease in the spinal fluid total protein and albumin fractions possibly reflects the decreased serum albumin level resulting from persistent proteinuria. The slight increase in the alpha fractions may result from increased glycoprotein associated with this component.

Serum Cholesterol, Diet and Stress in Patients with Coronary Artery Disease. JAMES F. HAMMARSTEN, CHARLES W. CATHEY, ROBERT F. REDMOND, and STEWART WOLF,* Oklahoma City, Okla.

Careful measurements of serum cholesterol were made each week for an average of 9 and up to 14 months in a group of 12 male subjects (ages 30 to 70) who had survived a well documented myocardial infarction. The method gave reproducibility in duplicate samples within 2 per cent and recoveries better than 98 per cent. Each subject kept a daily record of what he ate. The diets were relatively high in cholesterol and fat content and were maintained at the same level throughout the period of study. There were no significant changes in body weight and no gross changes in the amount of exercise performed. A strong and positive patient-physician relationship developed but no therapeutic agents were administered. Nevertheless in every patient a decrease in serum cholesterol was observed over the 9 months of the study. The mean decrease for the group was 13 per cent ($p = 0.001$).

Independently of the chemical determinations, each patient was studied carefully from the standpoint of his life adjustment and his reaction to people and events in his day-by-day experiences, and judgements covering each week were recorded. In addition to the over-all downward trend of serum cholesterol, there were significant variations from week to week. The average intra-individual range was 103 mg. per cent. On 20 occasions serum cholesterol rose higher than the mean value for that individual by more than 15 per cent. It was striking that 19 of the 20 occasions of high cholesterol corresponded with periods that had been separately judged as particularly stressful for the individuals concerned.

The Cellular Basis for the Electrocardiographic Changes Associated with Alterations in Serum Potassium. HANS H. HECHT,* JOSEPH M. HEATH, EUGENE S. MAIER, RAMON L. LANGE, and CLIFFORD V. NELSON, Salt Lake City, Utah.

The characteristic changes in the electrocardiogram of patients with altered extracellular potassium concentration provided a means by which the role of K^+ in the genesis of cardiac action potentials and its role in cardiac automaticity may be assessed. This can be approached in two ways: 1) Assuming the surface electrocardiogram to represent membrane *current*, a monophasic curve may be constructed by double integration, which, though nonquantitative, should resemble the average time course of transmembrane *potential* under circumstances of abnormal K^+ concentrations. 2) Transmembrane potentials may be recorded directly by microelectrodes from isolated spontaneously beating hearts (bull frog) perfused

with Tyrode solution of variable potassium content but unchanged in osmolarity.

The two approaches were carried out and yielded complementary information. Low potassium perfusion (increase in intracellular: extracellular gradient) resulted in prolongation of action potential duration and a more gradual than normal restoration of resting potential in diastole. This agreed with the double integration analysis and coincided with flattening of T-waves, and large U-waves in surface potentials in man, but without demonstrable U-waves in frogs. The time relationships suggest, however, that under these circumstances the appearance of U-waves is causally related to the last phase of ventricular recovery. Increasing extracellular K^+ concentration, and thereby decreasing the K^+ gradient, resulted in decrease in resting membrane—and action—potential, shortening of its duration, and steepening of the final phase of recovery in the experiments with microelectrodes. The geometric analysis suggested that the conduction disturbances and QRS changes of the surface record in hyperpotassemia are the result of slowed cellular depolarization caused by a decrease in resting membrane potentials. The large hyperpotassemic T-waves constitute a summated effect of an increased rate of recovery. Both the geometric and the experimental analyses are consistent with an excessive cellular outflow of K^+ in hypokaliemic states and a smaller than normal flux in hyperpotassemia. By inference it may be stated that, as in nerve, intracellular accumulation of K^+ is linked to the magnitude of resting membrane potentials of the ventricular myocardium and that this ion is concerned with cardiac recovery from the excited state.

Intestinal Excretion of Cholesterol: A Mechanism for Regulation of Plasma Levels. LEON HELLMAN,* ROBERT S. ROSENFELD, WILLIAM INSULL, JR., and EDWARD H. AHRENS, JR.,* New York, N. Y.

A physiologic mechanism by which the plasma cholesterol level can be elevated or reduced has been elucidated by means of a "labelled sterol balance study." This was performed following intravenous administration of cholesterol-4-C^{14} to a patient with hyperlipemia and xanthoma tuberosum. Since *in vivo* this radiocarbon atom remains permanently fixed in the sterol molecule, measurements of excreted radioactivity accurately reflect net changes in the excretion of cholesterol and its transformation products. The following observations were made:

1. Plasma and fecal cholesterol specific activities approximate each other, thus indicating that fecal cholesterol was largely derived from a pool in isotopic equilibrium with plasma sterol.

2. Four weeks after administration of the labelled cholesterol, the patient's diet was changed from *ad libitum* to a 40 per cent butter oil formula. Plasma cholesterol rose from 478 to 720 mg. (net gain 7.2 gm.) and fecal excretion of cholesterol and its end-products decreased by a minimum of 5.4 gm. during this period.

3. When the butter diet was replaced five weeks later by an isocaloric 40 per cent corn oil formula, cholesterol fell from 720 to 340 mg. (net loss 11.4 gm.) and fecal excretion of cholesterol and its end-products increased by a minimum of 9.6 gm.

4. The plasma cholesterol specific activity curve was similar to that observed in normocholesteremic subjects. No changes in slope were seen in the successive dietary periods.

These novel findings were made possible by purposeful study of *unsteady states* when plasma lipide concentrations were altered by simple dietary manipulations. The data indicate that the character of the dietary lipides affects the quantity of plasma cholesterol by diminishing or increasing the fecal excretion of cholesterol and its transformation products. Basis is provided for an interpretation of hypercholesteremic states in man in which attention will be focussed primarily on intestinal excretory mechanisms.

Alterations in Phosphorus Metabolism During General Anesthesia in Man. DOROTHY H. HENNEMAN and LEROY D. VANDAM, Boston, Mass. (Introduced by Francis D. Moore).

Previously observed changes in peripheral blood concentrations of carbohydrate metabolites during ether and thiopental anesthesia in normal man have been investigated further by the administration of epinephrine (0.01 mgm. per Kg. subcutaneous) and insulin (0.1 unit per Kg. intravenous).

Epinephrine with either agent increased blood glucose more than in unanesthetized subjects and to levels 150 mgm. per 100 ml. above those expected with ether alone. Elevations in organic acid concentrations (pyruvic, lactic, citric) were no more than those after epinephrine without anesthesia, or during ether anesthesia alone. Inorganic phosphorus concentrations decreased 0.1 to 0.4 mgm. per 100 ml. thirty minutes after epinephrine, but thereafter, unlike the expected unanesthetized response, steadily increased 1 to 2 mgm. per 100 ml.

Insulin during ether initially lowered blood glucose an average of 25 mgm. per 100 ml., but concentrations remained at hyperglycemic levels throughout the three-hour test period. Inorganic phosphorus levels rose after insulin rather than decreasing sharply as in the unanesthetized subject. Organic acid changes induced by insulin were no more than those expected with ether alone and after insulin in the unanesthetized patient.

The elevations in serum inorganic phosphorus are consistently associated with abnormal hyperglycemic responses to administered glucose, epinephrine, and insulin using either anesthetic agent. These abnormal responses are not associated with abnormally increased elevations in lactate; total lactate changes under these experimental conditions are never more than those expected with ether anesthesia alone. The lactate and pyruvate changes with ether might be related to an increased glycogenolysis peculiar to ether, but unrelated to the observed phosphorus changes. We suggest that ether and thiopental directly alter phosphorylating mechanisms associated with

glucose utilization, rather than directly inhibiting glucose oxidation.

Two Mechanisms of Sustained Hypercalcemia Following Hypervitaminosis D and the Milk-Alkali Syndrome. PHILIP H. HENNEMAN and WILLIAM H. BAKER, Boston, Mass. (Introduced by Paul C. Zamecnik).

It is well known that hypercalcemia, with its attendant depression of renal function, may persist for three to twelve months following withdrawal of vitamin D in hypervitaminosis D and following the discontinuation of milk in the hypercalcemia syndrome resulting from excessive milk and alkali ingestion. A complete balance study is presented which illustrates increased absorption of calcium continuing three months after cessation of excessive vitamin D therapy. Storage of vitamin D, probably in the liver, as is known to occur in other species, presumably accounts for the persistent hypercalcemia of such patients.

A complete balance study in a second patient, performed five months after discontinuation of excessive vitamin D intake, illustrates persistent hypercalcemia not due to increased calcium absorption. Calcium^{45} has been administered intravenously to this patient and to two patients with visible metastatic calcification due to excessive milk and alkali intake. The rate of calcium^{45} disappearance from the blood was greatly accelerated in all three patients. This rapid decline of blood specific activity was not due to increased excretion. The calcium "pool" (per cent of calcium retained in the body/specific activity of serum) at nine days was increased from normal values of 10 to 20 gms. to 42 gms. in the patient with hypervitaminosis D and to 112 gms. in one of the patients with metastatic calcification. Soft tissue calcification, which may or may not be visible by X-ray during life, presumably accounts for this increase in exchangeable calcium. It is suggested that such soft tissue calcification is produced at high serum calcium levels. Following withdrawal of milk or vitamin D, resorption of these soft tissue deposits of calcium may be sufficiently rapid to maintain hypercalcemia for prolonged periods.

The Suppression of Hyperadrenocorticism by Amphenone in Man. R. HERTZ, D. M. BERGENSTAL,* H. A. LUBS, and S. J. JACKSON, Bethesda, Md.

Amphenone (1,2-bis(p-aminophenyl) 2-methyl-1 dihydrochloride) produces an abrupt and reversible suppression of corticoid output in eucorticoid patients as well as in cases of Cushing's syndrome attributable either to adrenal cancer (7 cases) or adrenal hyperplasia (2 cases). This suppression is manifested by: (a) decrease in both plasma and urinary corticoids, (b) reduction in blood pressure, (c) reduction in steroid diabetes, (d) control of mental aberrations attributable to excessive endogenous corticoids, (e) a variable decrease in urinary 17-ketosteroids. This suppression of corticoid output is promptly reversed upon cessation of amphenone administration. In two cases an excessive rebound effect was manifested by extreme exacerbation of hyperadrenocorticism.

In these brief courses of amphenone treatment ranging from one to 32 days, no significant effect upon the clinical progression of adrenal carcinoma was noted.

The toxic effects of amphenone, notably somnolence, drug rash, and methemoglobinemia, limit its clinical application to short-term use either for diagnostic purposes or for the acute control of the clinical and metabolic features of hyperadrenocorticism.

The Pentose Phosphate Pathway in Human Metabolism. HOWARD H. HIATT, Boston, Mass. (Introduced by H. L. Blumgart).

Although there is now much evidence demonstrating the importance of the pentose phosphate pathway (PPP; hexose monophosphate shunt) in many biological processes, no information exists concerning its function in man. The role of the PPP in human metabolism was explored in two ways: 1) a study of the mechanism of ribose synthesis in a human carcinoma cell (strain HeLa) grown in tissue culture; and, 2) a study of the effects and fate of D-ribose given human subjects.

Degradation of nucleic acid ribose isolated from HeLa cell grown in the presence of glucose-1-C^{14} revealed radioactivity mainly in the first carbon atom of the ribose molecule; ribose from cells exposed to glucose-2-C^{14} was predominantly labeled in carbons 1 and 2. Such isotope distribution is consistent with ribose synthesis via the PPP.

Twenty-four patients without liver disease infused intravenously with D-ribose, 200 or 400 mg. per Kg. body weight, had half-times of ribose disappearance of 18 ± 2, or 26 ± 3 minutes, respectively. Half-times of 31, 33, 35, and 47 minutes were observed in four subjects with hepatic insufficiency. Urinary excretion averaged 8 per cent of the infused sugar, and an average volume of distribution of 28 per cent of body weight was found. Ribose disappearance was accompanied by an average decrease in blood glucose of 33 per cent, and in six of ten subjects by a fall in plasma inorganic phosphate.

The mechanism of D-ribose assimilation was studied by isolating and degrading liver glycogen glucose from mice given ribose-1-C^{14} intraperitoneally. Radioactivity was found primarily in glucose carbons 1 and 3. Of the known reactions in the intermediary metabolism of carbohydrate only those of the PPP can explain this isotope distribution.

The reactions of the pentose phosphate pathway responsible for ribose biosynthesis in man and for ribose metabolism in mammals will be discussed.

Estimation of the Retinal Venous Blood Oxygen in Man by Photographic Means. J. B. HICKAM* and R. FRAYSER, Durham, N. C.

Many pathologic changes in the optic fundus, including some which occur in systemic disorders, are ascribed to circulatory insufficiency, but sometimes with

tenuous evidence. For evaluating the role of retinal vascular insufficiency in various retinopathies, it would be highly desirable to have a means of measuring the per cent oxygen saturation in the retinal venous blood of man. The present communication describes the development of a method for accomplishing this by means of fundus photography.

The method employs the principle that per cent oxygen saturation of blood can be related to the relative intensities of reflected red and infra-red light. Relative light intensities can be measured from the density of the images produced on infra-red sensitive film. With blood in a cuvette, the per cent oxygen saturation of thirty-nine samples from nine subjects, ranging from 100 to 50 per cent saturation, was photographically measured, using the fundus camera. Oxygen saturation bore a linear relation to the difference between film densities produced by reflected red and infra-red light, with a standard deviation from regression of 4.5 per cent saturation for the original negatives and 6.4 per cent for 8-diameter enlargements. In the optic fundus itself, measurements have been made on enlarged images of the retinal vessels as they cross the disc. With such thin vessels on a near-white reflecting background, calculations appropriate to transmitted light can be applied. The method is being calibrated on retinal arteries, the blood oxygen saturation of which can be varied within wide limits by having the subject breathe different gas mixtures. On four normal subjects, the per cent oxygen saturation of retinal venous blood has averaged 69 per cent (range 60 to 75 per cent). Rapidly reducing arterial oxygen saturation by about 25 per cent causes almost as great a reduction in venous blood oxygen concentration. The method appears useful, and it is being applied to the study of appropriate problems.

The Effect of an Antibody to Properdin on Hemolysis of Erythrocytes from Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH). CARL F. HINZ, JR., Cleveland, O. (Introduced by Austin S. Weisberger).

Previous studies demonstrated that properdin, a normal serum protein, is required for the *in vitro* hemolysis by normal serum of erythrocytes from patients with PNH, but not for immune hemolytic reactions. Recently an antibody to human properdin (Anti-HP) has been produced in rabbits by Pillemer, Hinz, and Wurz. Anti-HP inactivates properdin isolated from serum or properdin in serum but does not inactivate complement. It is stable at 56° and inactivates properdin even at 1° C in the absence of complement. Anti-HP capable of inactivating 300 units of properdin per ml. of antiserum has been produced.

Addition of Anti-HP to normal human serum markedly decreases the ability of that human serum to hemolyze PNH cells *in vitro*, but has little effect on immune isohemolysis (Anti-A), which does not require properdin. Further addition of properdin to the human serum restores its hemolytic activity which can again be abolished by further addition of Anti-HP, thus demonstrating the specific effect both of properdin and Anti-HP. The effect of Anti-HP on PNH hemolysis is quantitatively similar to the effect of Anti-HP on properdin when measured in the zymosan assay. The inhibitory property of anti-HP against PNH hemolysis is removed by adsorption with properdin or the insoluble properdin-zymosan complex, but Anti-HP is not affected by adsorption with reagents which do not contain properdin. These observations confirm the specificity of the antibody.

This effect of an antibody specific for properdin confirms the requirement for properdin in PNH hemolysis, and presents a new means of studying the *in vitro* interactions of properdin with PNH erythrocytes and other substrates. It may eventually prove useful for studying *in vivo* the role of properdin in PNH hemolysis and in other reactions in which properdin participates.

A Plasma Factor That Protects Against the Lethal Action of Endotoxin. MONTO HO and EDWARD H. KASS,* Boston, Mass.

Because of indications that endotoxins play a role in the pathophysiology of many disease states, exploration of some mechanisms of resistance to these bacterial substances was undertaken. One such mechanism, hitherto undescribed, is presented.

1. The lethality of crude endotoxins from Gram negative bacteria (*S. typhi* Ty 2, *S. typhi* 0901 and *E. coli* 0127:B8), prepared by killing bacterial suspensions with chloroform, was attenuated when these endotoxins were incubated with normal human plasma fractions and injected intracardially into rats. The protective effect was still demonstrable when the endotoxin of chloroform-killed *S. typhi* Ty 2 was partially purified by trypsin digestion, and alcohol and ether precipitations. However, there was no protection against highly purified endotoxins prepared by the Boivin trichloracetic acid extraction method from the same organisms.

2. Protection against intracardiac injection of the partially purified Ty 2 endotoxin was also obtained with rabbit and rat plasmas. In human plasma, this protective effect was most marked in Fractions III and IV (Cohn), and minimal or absent in Fractions I, II and V. Significant protection was evident with active fractions equivalent to as little as 0.025 ml. of plasma.

3. The plasma protective factor was non-dialyzable, stable at 60° C for 30 minutes at pH 7, and resistant to repeated freezing and thawing, but was destroyed by boiling. Solutions of pure properdin, in amounts comparable to that found in the plasma fractions used, gave little or no protection. The protective factor was also independent of antibody.

4. Preliminary observations suggest that the inactivation of endotoxin by plasma fractions may be enzymic. The data indicate a mechanism of resistance against a crude bacterial product such as may be found in natural infections. When this product is "purified" by certain methods, the protective mechanism herein described is no longer active.

The Effect of Induced Hypocalcemia on Urinary Phosphorus Excretion in Dogs. JAMES F. HOLLAND, ROBERT BRENNER, ELTON ROCK, and ETHEL DANIELSON, Buffalo, N. Y. (Introduced by David K. Miller).

Suppression of parathyroid secretion was postulated by Howard *et al.* when they demonstrated decrease in urinary phosphorus excretion after induced hypercalcemia in man. We had undertaken administration of ethylene diamine tetra acetate (EDTA) infusions (pH 7.4) to dogs to demonstrate a possible hypocalcemic stimulus to parathyroid secretion.

Decrements in serum calcium concentration of 1.2 to 6.0 mg. per cent (median 3.6) were produced in 15 experiments in 12 normal dogs immediately after EDTA infusion of 65 to 125 mg. per Kg. (median 100) lasting 5 to 17 minutes (median 10). Glomerular filtration rate (GFR) measured after initial load and sustaining infusion of creatinine was essentially constant throughout each experiment.

The per cent of filtered phosphorus reabsorbed in 9 experiments on 8 dogs was decreased 6.5 to 32.2 per cent below baseline (median 20.0 per cent). Inhibition of phosphorus reabsorption began in 20 to 40 minutes and was maximal in 40 to 140 minutes. Maximal phosphate excretion was 0.2 to 0.55 mg. per min. (median 0.35) above baseline. In 6 experiments on 5 dogs (one dog from above group) phosphorus reabsorption increased 3.8 to 17.8 per cent (median 9.8) above baseline and excretion decreased. Amount of fall in calcium and variations in GFR were apparently unrelated to amount or direction of change in phosphorus reabsorption. Six experiments on 4 thyroparathyroidectomized animals gave inconclusive results.

The data demonstrate that increased phosphorus excretion occurs in a majority of instances after acute hypocalcemia induced by EDTA. The failure of some dogs to react in this manner is not explained. Possible mechanisms for decreased tubular reabsorption of phosphorus include stimulation of parathyroid secretion or effect of EDTA *per se* on tubular epithelium.

Further Studies in Electrolyte and Water Metabolism in Cardiac Patients Without Peripheral Edema. W. HOLLANDER and A. V. CHOBANIAN, Boston, Mass. (Introduced by Francis C. Lowell).

Previous studies have shown that renal capacity to excrete sodium is not necessarily impaired in congestive heart failure. The present study was undertaken to determine whether any alteration in electrolyte and water metabolism could be detected by radioisotope dilution techniques in heart failure prior to an impairment of renal excretory capacity.

Twelve cardiac patients with symptoms but no striking signs of congestive heart failure were studied. Most of them (but not all) had a reduced cardiac output, a prolonged circulation time, or a slight elevation of venous pressure. The exchangeable body sodium (Na^{24} space) and extracellular fluid volume ($S^{35}O_4$ space), as related to body weight, were significantly increased in this group whereas the exchangeable body potassium (K^{42} space) was normal as compared with 15 control subjects. Even though their total body sodium and extracellular fluid volume were increased, the cardiac patients were able to tolerate a daily 10-gram salt diet without developing a change in symptoms, an increase in weight, or retention of sodium. Furthermore, when rapidly infused with 300 cc. of 5 per cent sodium chloride, they excreted significantly *more,* rather than less, sodium than the control subjects even though their renal plasma flow (PAH clearance) and glomerular filtration rates (inulin clearance) were significantly reduced.

It was concluded that: 1. An increase in body sodium and extracellular fluid volume occurs in congestive failure prior to impairment of renal capacity to excrete sodium. 2. Early in congestive heart failure a new equilibrium of expanded extracellular fluid volume apparently is established under which sodium and water excretion are homeostatically controlled.

Pulmonary and Cardiac Blood Volumes of Patients With and Without Heart Disease. REX L. HUFF,* DANIEL PARRISH, and WAYNE CROCKETT, Seattle, Wash.

The amount of blood in the lungs, heart, and vessels between the lungs and heart was found to be 15.8 per cent of the total blood volume in recumbent patients without heart disease; when the formula, $V = F\ (\overline{T}_2 - \overline{T}_1)$, ($V$ = Volume, F = Cardiac Output, and $\overline{T}_2$ is the mean transit time recorded at a point distal to the mean transit time, $\overline{T}_1$, at another point), was applied to data curves obtained by simultaneous recording of I^{131} HSA transients, detected with well-collimated crystals placed over the anterior thoracic wall. The values obtained in this manner are much less than those of more difficult clinically applicable techniques, and approach those obtained in animals by direct volume measurement after appropriate simultaneous double ligations. About 0.65 of the blood in the heart and lungs exists between the pulmonary capillaries and aorta. In patients with essential hypertension this value may exceed 0.80 in the absence of congestive heart failure. The time-radioactivity curves over the lung are nearly always "pure" as are those from over the left ventricle; however, those curves from the regions of the right atrium and pulmonary artery are occasionally compound. Nevertheless, extrapolation to form curves representing single chambers or channels is readily performed. Cardiac output values calculated from the *in vivo* data are nearly identical from each of the four locations. With the appropriate placement of detectors the location as well as amount of abnormal accumulation of blood may be ascertained. This technique, requiring only a single intravenous injection and two venous samples, gives much information of value in understanding and managing cardiac disorders.

Gallop Rhythm in Mitral Valve Disease. HERBERT HULTGREN and THOMAS LEO, San Francisco, Calif. (Introduced by David Rytand).

Since gallop rhythm is favored by rapid ventricular filling, gallop sounds should be rare in tight mitral stenosis and frequent in mitral insufficiency. The presence of a gallop may therefore indicate a mitral valve unsuitable for valvulotomy. To examine this possibility, a phonocardiographic study has been made of 22 patients with tight mitral stenosis, 12 patients with combined stenosis and insufficiency, and 4 patients with pure mitral insufficiency. All diagnoses were established at surgery, necropsy or both. Patients were adults, and none had acute carditis. A Sanborn Twin Beam phonocardiograph was employed, and simultaneous electrocardiograms, apex impulses and carotid pulses were recorded for timing purposes. The following observations were made:

1. No patients with tight mitral stenosis exhibited gallop sounds. In 3 patients with a mild degree of stenosis faint third sounds could be identified.

2. All patients with combined stenosis and insufficiency had prominent early diastolic gallops initiating short diastolic murmurs. The gallops were preceded by outward precordial motion which was swift when the gallops were loud, and slower when gallops were faint. The gallop sounds were often difficult to identify clinically because of their variable intensity, their low frequency and their proximity to the diastolic murmur. Two of these patients also had presystolic gallops. Absence of pulmonary hypertension and maximal intensity at the apex indicated a left ventricular origin of these presystolic sounds which may be mistaken clinically for presystolic murmurs.

3. All patients with pure mitral insufficiency had early diastolic gallops, but diastolic murmurs were faint or absent.

Left ventricular gallop sounds, because of their absence in tight mitral stenosis and presence in combined lesions and pure mitral insufficiency, are an important sign of significant mitral regurgitation. Since accurate identification by auscultation may be difficult, phonocardiography should be employed when mitral insufficiency is suspected.

Evidence for an Accessory Pathway of Galactose Metabolism in Man. KURT J. ISSELBACHER, Boston, Mass. (Introduced by Mandel E. Cohen).

It has been demonstrated that the disease galactosemia is due to the congenital absence of a specific enzyme involved in the conversion of galactose to glucose. This enzyme, P-Gal transferase, is known to catalyze the following reaction:

(I) Gal-1-phosphate + Uridine diphosphate glucose (UDPGlucose) $\rightleftharpoons$ UDPGalactose + Glucose-1-phosphate

However, recent studies with C^{14}-galactose in an adult patient with galactosemia have shown conclusively that some conversion of galactose to glucose does occur in this disease in spite of the known enzymatic defect. This suggested that in all likelihood there must exist another pathway for forming UDPGalactose than that indicated by reaction I. Evidence has now been obtained by means of a cell-free preparation of mammalian liver and the use of C^{14}-galactose-1-phosphate that there exists another route for the synthesis of UDPGalactose. The reaction has been shown to involve uridine triphosphate (UTP) and to proceed according to the following equation:

(II) Galactose-1-phosphate + UTP $\rightleftharpoons$ UDPGalactose + PP (pyrophosphate)

The enzyme involved is found in the nuclei of liver cells and is called "UDPGalactose pyrophosphorylase." The UDPGalactose formed via this pathway is then converted to UDPGlucose and in turn to glucose-1-phosphate by means of reactions previously described.

Although normally the liver contains much less pyrophosphorylase than transferase activity, it would appear that the existence of this additional pathway (II) could effectively account for the metabolism of galactose which occurs in galactosemia. The overall significance of these two pathways in normal individuals remains to be elucidated.

Characteristics of a Previously Unidentified Protein Present in Normal Rat Serum. RALPH F. JACOX,* Rochester, N. Y.

Rat sera were separated electrophoretically utilizing a starch block technique. Electrophoresis was made for a period of 20 hours in 0.05 M barbital buffer at pH 8.6, with current of 450 volts and 20 milliamperes. One-centimeter sections of the starch block were then eluted with 0.08 M sodium chloride solution. Each eluate obtained was sub-fractionated by a cationic detergent technique (Jacox, R. F., J. Clin. Invest., 1953, 32, 661) to determine the distribution of components reacting with cationic detergent as alpha globulins, beta-gamma globulins and albumin. By these methods, a previously undescribed serum protein component has been found which has the following characteristics: 1) The new serum fraction migrates more rapidly than albumin and causes an asymmetrical appearance to the albumin peak. 2) It precipitates with 48 per cent saturation of $(NH_4)_2SO_4$ and thus has characteristics of a globulin; by this criterion it is not a mucoprotein. 3) The rapidly migrating "globulin" is present in serum in nearly the same concentration as albumin. 4) It reacts with cationic detergent at pH 6.65, whereas albumin will not precipitate with cationic detergent at pH 9.5.

Thus, this fastest migrating electrophoretic component of rat serum has many characteristics of an alpha globulin.

Normal rats were given 20 mgms. of cortisone subcutaneously each day for 14 days. The serum protein values were determined daily by the cationic detergent method. A striking fall of the "alpha globulin" reacting component and an increase of albumin were observed after seven days of cortisone administration. The sera of cor-

tisone-treated rats were then analyzed by starch electrophoresis followed by sub-fractionation with cationic detergent. A *decrease* of the rapidly migrating "globulin" and an *increase* in albumin concentration were observed, suggesting that some interconversion of these components may occur during cortisone intoxication.

Cellular and Extracellular Alterations in Heart Failure: Studies During Diuresis. JOHN R. JAENIKE and CHRISTINE WATERHOUSE,* Rochester, N. Y.

The significance of cellular changes in water and electrolyte composition in congestive heart failure has been a controversial issue. This question is particularly pertinent in the evaluation of hyponatremia accompanying severe failure. For this reason detailed metabolic studies have been conducted during diuresis in patients with heart disease of varying severity, utilizing nitrogen, electrolyte, water, and caloric balances.

In all subjects studied, during either mercurial or spontaneous diuresis, chloride space contraction and total water loss agreed closely, indicating an insignificant loss of cell water. This observation was verified by six studies in which body water changes were measured directly by deuterium dilution, which correlated well with changes calculated from water and caloric balance.

Significant differences in electrolyte metabolism were observed between various patients, however. Subjects readily responsive to mercurials or undergoing spontaneous diuresis excreted primarily sodium chloride, showed negligible internal shifts of sodium, and variable changes in potassium balance, probably unrelated to the heart failure *per se*. All patients in this group showed a calculated decrease in osmotically active total body cation which either equalled or slightly exceeded external losses, the latter suggesting osmotic inactivation of cation.

Successful mercurial diuresis was accomplished in three patients with advanced cardiac disease, previously refractory to therapy. Diuresis was characterized by relatively large urinary losses of potassium, low urinary sodium to chloride ratios, a shift of sodium into cells, and correction of pre-existent hyponatremia in two subjects. Clinical improvement ensued in the hyponatremic patients, and restoration of normal serum sodium concentration was attributable to excretion of extracellular water in excess of sodium, rather than an increase in extracellular fluid sodium content.

In contrast to other patients, diuresis in the hyponatremic subjects was accompanied by changes which suggested mobilization of osmotically active cation or correction of a deranged osmotic equilibrium between cells and extracellular fluid.

The Effect of Nucleosides on Osmotic Resistance of Mammalian Erythrocytes in Relation to the Age of the Cells. ERNST R. JAFFÉ, GRACE A. VANDERHOFF, BERTRAM A. LOWY, and IRVING M. LONDON,* New York, N. Y.

In studies on the relationship of metabolic activity of the erythrocyte to its structural integrity, the resistance of fresh human erythrocytes to osmotic stress has been found to be enhanced by incubation of the erythrocytes with various purine ribosides. The following experiments were designed to determine the influence of the age of the mammalian erythrocyte upon this effect of purine ribosides.

Whole blood from a normal adult man was collected in acid-citrate dextrose solution and stored at 4° C. At intervals during the subsequent 168 days, aliquots of the stored erythrocytes were washed with isotonic sodium phosphate buffer, pH 7.3, and incubated in the buffer with and without nucleosides or glucose. After incubation the osmotic fragility was determined. The effect of adenosine or inosine in enhancing resistance to osmotic lysis was most marked initially and declined progressively; after 168 days no effect was demonstrable. The protective effects of glucose and deoxyadenosine were minimal.

Adult male rabbits received glycine-2-C^{14} for labeling of newly formed erythrocytes. At intervals of 4, 8, 13, 46, and 60 days after injection of the labeled glycine, one of the rabbits was exsanguinated and the washed erythrocytes were incubated in isotonic sodium phosphate buffer, pH 7.3, with and without added nucleosides. After exposure to hypotonic phosphate buffer solutions, the extent of hemolysis and the radioactivity of the hemin isolated from the hemoglobin of the lysed cells were determined. It was found that young rabbit erythrocytes are more resistant to osmotic stress than older cells and that the effect of inosine in enhancing resistance to osmotic lysis is much more pronounced in young than in old erythrocytes.

The finding that the ability of purine ribosides to enhance osmotic resistance is more pronounced in young than in old erythrocytes is pertinent to the problems of erythrocyte preservation and of the mechanism of aging of erythrocytes *in vivo*.

Serum Atropinase. B. V. JAGER,* Salt Lake City, Utah.

Atropinase (tropinesterase), an enzyme present in the serum of certain rabbits, hydrolyzes atropine into pharmacologically inert tropine and tropic acid. The amount of enzyme present in the serum is determined by a manometric procedure, using atropine sulfate as a substrate. A related enzyme producing hydrolysis of atropine is found in the livers of many kinds of animals. In the present study, serum obtained from a wide variety of animal species was examined for the presence or absence of serum atropinase. Some types of rodents other than rabbits were found to have this enzyme in the serum. Striking differences were noted when the effects of atropine were compared in rabbits which had serum atropinase and in rabbits without this enzyme. This has lead to the development of a simple technic for detecting the presence or absence of atropinase in the serum of rabbits. This has been examined in over 100 rabbits.

Vitamin B_{12} Activity of Leukemic Sera and Tissues. G. WATSON JAMES, III * and LOIS RISKIN, Richmond, Va.

The unicellular chrysomonad, *Poteriochromonas stipitata,* like *O. malhemensis* is considered to have a specific growth requirement for cyanocobalamin and differs in this respect from *Euglena gracilis,* the usual assay organism. In 30 normal individuals the vitamin B_{12} activity of the serum assayed 0 to 275 $\mu\mu$g. per ml. (mean = 44 $\mu\mu$g. per ml., standard error of mean = 11); 15 patients with chronic granulocytic leukemia had levels of 100 to 11,750 $\mu\mu$g. per ml. (mean = 1676 $\mu\mu$g. per ml., standard error of mean = 194); 14 patients with acute leukemia (not terminal) assayed from 0 to 132 $\mu\mu$g. per ml. (mean = 29 $\mu\mu$g. per ml. with standard error of mean = 4). Five patients with untreated pernicious anemia had less than 1 $\mu\mu$g. per ml. vitamin B_{12} activity. Patients with various hematologic disorders and those with pernicious anemia under treatment were not appreciably different from normal individuals.

In the same sera, comparison of two assay methods demonstrated in ten normal subjects that the *Euglena gracilis* assay (304 to 728 $\mu\mu$g. per ml., mean = 455 $\mu\mu$g. per ml.) was six times greater than that for *P. stipitata;* however, in nine chronic granulocytic leukemia patients the *Euglena* assay (370 to 11,533 $\mu\mu$g. per ml., mean = 3655 $\mu\mu$g. per ml.) was roughly twice that of the *P. stipitata.* Other hematologic disorders were similar to the normals. When leukemic sera and normal sera are assayed with *P. stipitata* using amounts of extract representing 0.05, 0.1, 0.2, and 0.4 ml. of serum a striking difference is observed. Normal sera, sera from acute leukemia patients and subjects with various hematologic disorders showed no significant difference in total calculated values ($\mu\mu$g. per ml.) at the four different serum levels, whereas in the leukemic sera the assay value increased with increasing amounts of serum.

Vitamin B_{12} activity of liver, kidney and spleen from leukemia patients and normal individuals decreased as the amount of tissue content increased suggesting the presence of tissue inhibitor substance to the growth of *P. stipitata.*

Pyrogenic and Leukopenic Effects of Immune Hemolysis in Man. JAMES H. JANDL, Boston, Mass. (Introduced by W. B. Castle).

Chills, fever, and alterations of leukocyte levels are frequently observed during episodes of hemolysis. Injections of small amounts of washed, Cr^{51}-labelled ABO-incompatible red cells into normal subjects caused an abrupt, profound fall in circulating granulocytes and monocytes, coinciding with the sequestration of the labelled red cells and the onset of hemoglobinemia. Within 20 minutes an immature granulocytosis appeared, followed by an adult granulocytosis, lymphopenia, and occasionally mild fever (average rise: 0.5°F). Comparable preparations of type D red cells injected into D-sensitized subjects caused a similarly profound but more slowly developing and more prolonged leukopenia, which again coincided in onset with the slower rate of red cell destruction. In these subjects marked pyrogenic responses occurred invariably, consisting of initial hypothermia, followed by chills appearing at the time of extreme leukopenia, and then fever (average rise: 3.0°F) associated with an immature granulocytosis and lymphopenia. Injection into normal subjects of type D red cells sensitized *in vitro* with incomplete anti-D serum produced almost identical phenomena. Neither fever nor leukopenia followed injections of hemoglobin, whole blood hemolysates, or soluble red cell antigens, or the destruction of red cells *in vivo* by non-immune mechanisms.

In vitro, anti-D sensitized red cells, unlike normal cells, were observed to cluster tightly around individual granulocytes, particularly those of immunized subjects, and upon adhering, to become spherocytes.

These studies indicate that the sequestration of red cells by immune mechanisms in man is attended by leukopenia (leukocyte destruction?) and the release of "endogenous pyrogen." The effects of red cell destruction by incomplete and by complete antibodies, respectively, are analogous to the effects described by others of bacterial pyrogens on normal and "tolerant" animals. While being themselves destroyed, granulocytes are seemingly involved in the spherocytosis and hemolysis of red cells altered by antibodies *in vivo.*

Mechanisms of Pancreatic Secretion of Electrolytes in Man: The Effects of Diamox® and Disease. HENRY D. JANOWITZ * and DAVID A. DREILING, New York, N. Y.

The relationship between rate of flow and electrolyte composition of pancreatic juice stimulated by secretin or inhibited by Diamox® was determined in 65 normal subjects during continuous aspiration of duodenal contents free of gastric contamination. In these individuals, HCO_3 concentration (25 to 151 mEq. per L.) varied directly with rate of secretion, Cl varied inversely, and the sum (HCO_3 + Cl) remained constant (155 to 157 mEq. per L.), approaching the osmolarity of the plasma. Pancreatic excretion of Na (139 to 143 mEq. per L.) and K (6 to 9 mEq. per L.) was independent of the rate of flow. When the rate of secretion was inhibited by i.v. administered Diamox® (50 to 121 mg. per Kg. body weight) in 14 normal subjects, the maximum HCO_3 concentration tended to remain constant despite marked reduction of volume flow (mean inhibition = 69 per cent) and HCO_3 output (mean inhibition = 72 per cent). These changes were independent of plasma electrolyte alterations. The concentrations of Na, K, and the sum (HCO_3 + Cl) were unaltered.

These relationships held for 61 patients with pancreatitis except that they were unable to achieve as high HCO_3 concentrations at comparable rates of flow (max. 81 mEq. per L.).

Previous investigators have hypothesized that the elec-

trolyte composition of pancreatic juice at varying rates of flow represents the admixture of an isotonic HCO_3 solution with one resembling interstitial fluid. The present observations indicate that carbonic anhydrase activity is required for the elaboration of the major portion of the HCO_3 output of the human pancreas. They also suggest that the HCO_3/Cl ratio results from alteration of the primary secretion of isotonic HCO_3 by a process of limited capacity in which HCO_3 is exchanged for Cl, presumed to occur in the intercalated ducts, and that Diamox® interferes with this exchange.

Etiology of Epidemic Keratoconjunctivitis. ERNEST JAWETZ,* YUKIHIKO MITSUI, LAVELLE HANNA, PHILLIPS THYGESON, and SAMUEL KIMURA, San Francisco, Calif. and Kumamoto, Japan.

Epidemic keratoconjunctivitis (EKC) is a serious eye disease of well defined clinical characteristics. Following an acute inflammatory stage there develop round subepithelial opacities in the cornea which may persist up to two years and seriously interfere with vision. EKC occurs both sporadically and in epidemics all over the world. Its etiology is not established. Claims have been made for the role played by different viruses but most of them have not been substantiated. Textbooks list a virus (Sanders) resembling St. Louis encephalitis virus as etiologic agent. The available strains of this virus bear no serological relationship to the disease as it has existed since 1951. Based on the evidence presented below it is suggested that adenovirus type 8 (APC 8) is regularly associated with EKC and is capable of causing this disease.

1. Six strains of APC 8 have been isolated to date, all from eyes, five of which were clinically definite EKC. No strains of this virus have been isolated from other sources. APC 8 differs biologically from other adenovirus types.

2. Among 147 patients diagnosed as EKC during 1951–56 in North America, Japan, Austria, Germany, Switzerland, and Italy, 115 (78.2 per cent) had neutralizing antibodies to APC 8 in a serum dilution of 1:10 or greater. Among 174 controls from the same geographic areas and similar age distribution only 13 (7.4 per cent) had such antibody titers.

3. Of 25 adequate paired sera from patients diagnosed EKC, 21 showed a four-fold or greater rise in neutralizing antibodies to APC 8 but not to other adenovirus types. APC 8 antibodies declined rapidly so that titers of 1:20 were not found later than two years after onset.

4. One eye of each of five volunteers was inoculated with APC 8. Four developed typical EKC; the fifth had APC 8 antibodies at the time of inoculation and remained well.

The uninoculated eye became involved in three volunteers six to eight days after the inoculated eye. APC 8 virus was re-isolated from lesions and the volunteers developed significant antibody titer rises.

The Response of Diffuse Hyperfunctioning Goiter to Thyrotropin. W. MCK. JEFFERIES, R. P. LEVY, L. W. KELLY, JR., and G. COOPER, Cleveland, O. (Introduced by W. H. Pritchard).

The change in 3-hour thyroidal I^{131} uptake and serum protein-bound iodine (PBI) before and 24 hours after an intramuscular injection of thyrotropin (TSH) has been shown to reflect the effect of this hormone upon rate of iodide uptake and rate of hormonal release, respectively. In order to learn more about the pathologic physiology of hyperthyroidism with diffuse goiter, the responses of patients with this disease to a single injection of TSH before and at intervals after treatment have been studied. Of 19 subjects tested before treatment, 6 had rises in either I^{131} uptake or PBI or both of a degree which was probably significant (an increase of over 15 per cent of the tracer in the 3-hour uptake and over 3 mcg. per 100 ml., respectively). The remainder showed no response. These findings indicate that the thyroids of at least some persons with this disease do not continuously function at a maximum rate. Furthermore, they reveal that some hyperfunctioning thyroids may be trapping iodide at a maximum rate without releasing hormone at a maximum rate, whereas others may be releasing hormone as rapidly as it is formed even though iodide is not being trapped at a maximum rate. Neither severity of symptoms nor presence of exophthalmos correlated well with the rate of I^{131} uptake, the level of PBI, or the type of response to TSH. Tests on 10 patients with recurrent Graves' Disease revealed a similar distribution of responses.

Of the apparently euthyroid patients tested up to 34 years after subtotal thyroidectomy and up to 5 years after I^{131} therapy, most showed evidence that the remaining thyroid tissue continued to function at a maximum rate, although a few had normal responses. Some patients after treatment for hyperthyroidism had normal I^{131} uptakes with low PBI's or elevated uptakes with normal PBI's, demonstrating that I^{131} uptake is not a reliable indication of the state of thyroid function in such circumstances.

Arterial Oxygen Unsaturation and Possible Mechanisms of Its Production in Sickle Cell Anemia. WALLACE N. JENSEN, DONALD L. RUCKNAGEL, and W. JAPE TAYLOR, Pittsburgh, Pa. (Introduced by I. Arthur Mirsky).

Oxygen unsaturation of arterial blood has been found in a majority of patients with sickle cell anemia, in contrast to the normal oxygen saturation found in other types of anemia. In 19 determinations of arterial oxygen saturation, performed at widely spaced time intervals in 11 patients with sickle cell anemia, the range of saturation was 72 to 97 per cent with a mean of 85 ± 7.1 per cent. There was no correlation between the degree of anemia and the arterial oxygen unsaturation. One hundred per cent oxygen was administered by mask to 7 patients with a prompt rise in arterial oxygen saturation to normal levels. The infusion of normal blood to

5 patients in amounts sufficient to produce a mean increment of 3.7 gm. per cent hemoglobin was associated with a mean rise in oxygen saturation from a pre-transfusion mean of 83 per cent to a post-transfusion mean level of 93 per cent ($p < 0.01$).

The complete arterial oxygen saturation observed with 100 per cent oxygen breathing indicates that the arterial oxygen unsaturation is not the result of a circulatory veno-arterial shunt. The increased arterial oxygen saturation following the transfusion of normal erythrocytes indicates that diffuse pulmonary disease is not, alone, sufficient to cause the unsaturation. Since arterial oxygen unsaturation does not exist in other uncomplicated types of anemia, an increased rate of blood flow through the lungs in sickle cell anemia cannot, *per se*, explain the arterial oxygen unsaturation. It appears that the combination of a short pulmonary circulation time plus either diffuse subclinical pulmonary disease or an erythrocytic defect in the mechanism for oxygen uptake in sickle anemia cells provides the basis for arterial unsaturation.

The Contractility of Actomyosin Bands Prepared from Human Heart Muscle. K. KAKO and G. CABRERA, St. Louis, Mo. (Introduced by R. J. Bing).

Previously published studies of the afterloaded contractions of acetomyosin bands prepared from dogs' hearts have revealed no difference between the fresh preparation and that obtained one hour after death. This finding suggested the possibility of studying the contractile properties of actomyosin prepared from human hearts obtained during post-mortem examination. Actomyosin was prepared from human hearts obtained from one to four hours following the death of the patient. The protein was extracted in the usual manner. Actomyosin bands were prepared in a trough by compression of surface spread films of the protein. ATP was added and contractions were recorded with a null seeking device with the bands swimming on the surface of the trough solution. All contractions were afterloaded and shortening of the bands was expressed as a function of the load per "specific nitrogen content" of the bands. The latter represents the amount of nitrogen in micrograms per one millimeter of band in resting length. The results indicate that actomyosin bands prepared from human heart muscle shortened from 20 to 40 per cent of their initial length. No difference in shortening of fibers prepared one to four hours after death of the patient was noticeable, and the addition of a cardiac glycoside (digoxin) had no effect on the shortening of the bands. The contractility of actomyosin prepared from human hearts in failure will be discussed.

The Effects of Muscular Exercise on Net Splanchnic Glucose Production (NSGP) in Man. ROBERT F. KIBLER and W. JAPE TAYLOR, Pittsburgh, Pa. (Introduced by Jonas E. Salk).

In the resting, fasting state both the arterial glucose level and the hepatic glucose production are relatively constant. It has been estimated that under these circumstances approximately 80 per cent of the hepatic glucose output is utilized by the brain and the remaining 20 per cent by other tissues, presumably muscle. Although never proven, it might be assumed that muscular work would be accompanied by an increased hepatic glucose production. Accordingly, the effect of moderate to severe exercise on the fasting NSGP has been studied in six control, hospital subjects.

Exercise was accomplished in the recumbent position by pedaling a bicycle ergometer. A constant level of exercise was performed by four subjects for 15 to 40 minutes, in three of whom the total oxygen consumption increased by 140 per cent. The two remaining subjects exercised at an increasing rate to the point of exhaustion which occurred at 30 and 40 minutes. NSGP was determined as the product of the BSP hepatic blood flow and hepatic venous-arterial glucose difference. Measurements were made at five-minute intervals during exercise and in one subject for a twenty-minute period following exercise.

At no time during exercise did the NSGP change significantly from the control value. Arterial blood glucose levels did not fall in three subjects and in the other three fell between 3 and 6 mgm. per cent. The hepatic blood flow, BSP clearance and splanchnic oxygen consumption showed no significant change.

From these data, it is concluded that whatever additional glucose the muscles utilize during exercise of the degree performed in these studies is derived from muscle stores alone; and that during exercise the brain remains the major consumer of the hepatic glucose production. Studies are in progress to obtain confirmatory evidence for this conclusion from measurements of femoral and cerebral arteriovenous glucose differences during exercise.

Effect of Mycobacteriophage on Mycobacteria In Vivo. VERNON KNIGHT* and ARTHUR WHITE, Nashville, Tenn.

We have observed that a mycobacterial bacteriophage, D-29, isolated by others, causes lysis of several strains of human tuberculosis as well as the non-virulent *M. tuberculosis*, ATCC 607. It was further found that homogenate of guinea pig spleen did not inactivate the phage *in vitro* and that phage propagation occurred in high concentrations of human serum. Moreover, active phage could be recovered as long as 24 hours following injection into mice. Possible effects of phage on susceptible bacteria are (1) induction of lysogenesis, (2) development of phage resistance without lysogenesis, or (3) destruction of bacterial inocula by phage lysis. Studies with diphtheria bacilli have shown that the induction of lysogenesis may be accompanied by development of toxigenicity (virulence) of a previously avirulent culture. From studies with choleraphage it appears that changes in virulence of the vibrio may occur in the course of natural infection, as a consequence of phage activity. Therefore, in the absence of known inactivating host

mechanisms, experiments were performed to examine the effect of mycobacteriophage on mycobacteria, *in vivo*. Mice were inoculated intravenously with cultures of *M. tuberculosis* as follows: $H37R_V$, $H37R_A$, human strain 4, ATCC 607, and phage resistant ATCC 607. Several schedules of parenteral phage dosage were employed and the course of infection was followed by observation of mortality rates, or by colony counts of splenic homogenates obtained at intervals during the infection. Studies of myobacteria recovered from treated animals did not reveal evidence of lysogenesis, new development of phage resistance, nor modification of infection by phage treatment. The originally phage resistant cultures of ATCC 607 retained this characteristic through animal passage. The implications of these studies will be discussed.

A Six-Month Evaluation of an Anabolic Drug, Norethandrolone, in Chronically Underweight Individuals. Ross C. Kory, Robert N. Watson, Matthew H. Bradley, and Bruno J. Peters, Milwaukee, Wisc. (Introduced by William W. Engstrom).

Norethandrolone, 17 α-ethyl-17-hydroxy-norandrostenone (Nilevar®), has been shown to be an effective anabolic agent in patients with acute illnesses. The present study is a long-term evaluation of this steroid in underweight but essentially healthy individuals.

A daily oral tablet containing either placebo, 25 mg., or 50 mg. of norethandrolone was administered by the "double blind" technique to 56 underweight individuals for three months. These included: a) 29 young factory workers, 11 female and 18 male; b) 17 elderly male members of a domiciliary unit; and c) 10 male hospitalized patients with stabilized pulmonary tuberculosis. The code was then broken, the placebos discontinued, and all were given the steroid for three additional months.

Statistically significant gains in weight were observed in all groups receiving norethandrolone ($p < 0.01$); these usually were accompanied by increases in appetite and sense of well-being. The average weight gain was two pounds per month. The maximum was 20 pounds in six months. No patient exhibited edema or other signs of fluid retention.

Undesirable clinical effects were not observed in the male patients but appeared in nine of the eleven females; and consisted of menorrhagia in two, acne in four, and amenorrhea in seven. These effects were reversed by reducing the dosage or discontinuing the drug.

Unexpected retention of bromsulfalein was observed in about three-fourths of the patients. Abnormal dye retention was considerable (up to 51 per cent), other liver function tests were entirely normal, no clinical signs of liver disease appeared, and the effect was reversible with discontinuance of the drug. Liver biopsies in six individuals with high dye retention were normal. While a relationship may exist, no correlation could be made between weight gain and dye retention. These observations require explanation and the long-term use of steroids of this nature carefully appraised.

Factors in the Genesis of Pulsus Bisferiens. Peter T. Kuo and Arthur F. Whereat, Philadelphia, Pa. (Introduced by William A. Jeffers).

In a patient with luetic aortitis, aortic insufficiency and dissecting aneurysm of the aorta, a bifid pulse was found that was limited to the left arm only. This pulse pattern appeared as a catheter was withdrawn from the aorta to a point just distal to the dissection. This led to the study of 14 other patients with pulsus bisferiens who suffered from a variety of conditions, including: aortic valvular disease, patent ductus arteriosus, and aneurysm of the sinus of Valsalva that had ruptured into the right ventricle. Arterial and left ventricular pressure curves of these patients were obtained by arterial and retrograde left ventricular catheterization, using a small plastic catheter inserted percutaneously through a thin-walled 20 or 21-gauge needle. In all these 14 patients a bifid pulse was recorded in both brachial vessels, while a curve with many high frequency vibrations was simultaneously recorded in the femoral or the carotid artery. Exploration of the arterial tree indicated that the subclavian-brachial system was particularly prone to develop a bisferious pulse, and that aortic stenosis *per se* contributed little or nothing to the production of this pulse pattern. In all the patients pulsus bisferiens was increased by exercise, epinephrine or nor-epinephrine infusion, or by mechanical compression of a peripheral artery. In all it was decreased, after a premature beat, during the Valsalva maneuver, by amyl nitrite inhalation, and by the onset of severe heart failure. It is concluded that pulsus bisferiens is primarily a peripheral manifestation of an increase in cardiac stroke volume and velocity of peripheral blood flow.

The Pharmacology of the Placebo. Louis Lasagna,* Baltimore, Md.

It is well known that placebos can mimic drugs in producing therapeutic benefit or untoward effects. Certain details of the pharmacology of placebos are less widely appreciated. Data collected in experiments on a variety of populations (134 post-partum patients, 34 tuberculous patients in a sanatorium, 31 patients in a chronic disease hospital, and 93 patients suffering from post-operative pain) indicate how closely placebo effects may at times resemble effects generally considered attributable only to "active" agents.

These phenomena include: 1) time-effect relationships, both in acute single dose experiments and in repeated daily administration of placebos, and 2) correlation between efficacy of placebos and severity of disease. In single dose experiments, for example, "peaking" of therapeutic effect can be observed with placebos, with an initial building up, and a subsequent waning, of effect over a period of hours. "Cumulative" effects are likewise seen with placebos, in that progressive increase in therapeutic benefit may occur with repeated daily doses. "Carry-over" effects have also occurred, with therapeutic effect persisting for variable periods of time after cessation of

placebo therapy. In treating post-operative and post-partum pain, morphine, aspirin, and placebo all appear less effective in relieving severe pain than in relieving mild pain. Thus with placebos, as with active drugs, one can show a negative correlation between severity of complaint and therapeutic efficacy.

The implications of these data for the interpretation of uncontrolled experiments and for the design of controlled experiments will be discussed.

Uric Acid Formation in Patients with Gout. The Incorporation of 4-amino-5-imidazolecarboxamide-6[13] into Uric Acid. LEONARD LASTER, J. E. SEEGMILLER, DEWITT STETTEN, JR., and LOIS V. LIDDLE, Bethesda, Md. (Introduced by John T. King).

From studies of the incorporation of glycine-N^{15} into uric acid, Benedict *et al.* concluded that some gouty patients may possess a "shunt pathway" for urate biosynthesis. We have studied the incorporation of the purine precursor 4-amino-5-imidazolecarboxamide-C^{13}, "AIC," into uric acid in normal and gouty persons in order to explore further the nature of the proposed "shunt."

In the normal human, fed AIC-C^{13} is incorporated more promptly into urinary uric acid and in larger amounts than is glycine-N^{15}, and in a pattern that implicates a rapid and slow conversion. AIC depresses the incorporation into uric acid of simultaneously administered glycine-N^{15} without affecting its incorporation into creatinine, suggesting that there is a regulatory mechanism for endogenous urate production.

In five gouty patients studied so far the extent of AIC-C^{13} incorporation into uric acid was greater than normal and was substantially greater in those patients who showed an excessive incorporation of glycine-N^{15} into uric acid. In all the patients the conversion of AIC-C^{13} to uric acid occurred by rapid and slow pathways. In addition AIC-C^{13} suppressed the incorporation of glycine-N^{15} fed simultaneously. Although the per cent suppression varied considerably in different gouty subjects, two of them still incorporated into uric acid about twice as much N^{15} from glycine as did normal subjects under similar conditions of AIC administration.

These results suggest that there may be a defective regulatory mechanism for endogenous purine synthesis in some gouty patients.

The Renal Transport of Hemoglobin in Renal Disease. WILLOUGHBY LATHEM, Pittsburgh, Pa. (Introduced by Jack D. Myers).

Physiologic and pathologic evidence suggests that the transport of protein molecules across both the glomerular membrane and renal tubules may be altered in renal disease. However, quantitative information regarding these processes is limited. In the present study the rate of transfer of hemoglobin molecules at these sites was measured in patients with a variety of chronic renal diseases and proteinuria.

Approximately 10 gm. of hemoglobin (4 per cent solution prepared by hemolyzing the subject's erythrocytes in distilled water) were administered intravenously over a period of 30 minutes. Plasma and urinary hemoglobin concentrations were determined every 15 to 30 minutes for two hours. The clearance of inulin (C_{In}) was measured concurrently.

A linear relationship between the rate of hemoglobin excretion (U_{hgb} V) and the plasma level (P_{hgb}) was obtained, indicating a constant tubular reabsorptive rate (T_{hgb}) at the levels of P_{hgb} examined (below 400 mgm. per cent). This permitted the following calculations:

Plasma cleared of hemoglobin at the glomerulus (C^{g}_{hgb})

$$= \frac{\Delta U_{hgb} V}{\Delta P_{hgb}} \text{ (ml. per min.)}$$

Transfer rate of hemoglobin across the glomerulus (load)

$$= \frac{\Delta U_{hgb} V}{\Delta P_{hgb}} \times P_{hgb} \text{ (mgm. per min.)}$$

Tubular hemoglobin reabsorption (T_{hgb})

$$= \frac{\Delta U_{hgb} V}{\Delta P_{hgb}} \times P_{hgb} - U_{hgb} V \text{ (mgm. per min.)}$$

(The expression Tm_{hgb} was not employed owing to uncertainty concerning glomerular activity and tubular saturation in diseased nephrons.)

In subjects with renal disease C^{g}_{hgb} averaged 3.4 ml. per min. (normal, 10.8); T_{hgb} averaged 5.0 ml. per min. (normal, 10.2); C^{g}_{hgb}/C_{In} averaged 0.034 (normal, 0.090); and T_{hgb} per 100 ml. filtrate averaged 4.6 mgm. (normal, 8.9). These changes were not correlated with the degree of proteinuria or with the severity of renal functional impairment.

These results indicate that, at levels of P_{hgb} obtained, the transglomerular and tubular transport of hemoglobin is reduced in renal disease, both in absolute and relative (to C_{In}) terms. These changes may be attributable to structural or metabolic alterations in the diseased kidney, to competitive activity with plasma proteins, or to both.

Effect of Specific Antigen on Release from Human Leucocytes of the Factor Concerned in Transfer of Delayed Hypersensitivity. H. S. LAWRENCE* and A. M. PAPPENHEIMER, JR., New York, N. Y.

It has been shown that the delayed tuberculin type of hypersensitivity may be transferred to nonsensitive human recipients by injection of leucocytes or leucocyte extracts obtained from the blood of sensitive donors. The transfer factor (TF) resists treatment with DN-ase, RN-ase and crystalline trypsin. We are now reporting evidence that TF may be liberated from sensitive leucocytes by interaction with specific antigen.

One ml. of packed leucocytes from a highly tuberculin sensitive donor was suspended in the donor's fresh serum (or plasma) and divided in two equal portions. Twenty-five gamma PPD were added to the test suspension; the other served as control. Both suspensions were aerated by gentle rotation in siliconized tubes for 1 hour at 37° C. After centrifugation, the frozen and thawed DN-ase-treated test and control cell extracts were injected into

each of 2 tuberculin negative recipients. The corresponding supernates were injected into 2 other recipients. One week after transfer the recipients were skin tested with old tuberculin. The results showed that contact with PPD caused the complete extraction of TF into the supernate serum or plasma and the PPD-treated cells failed to effect transfer of tuberculin sensitivity. No TF could be detected in the control supernate.

The above experiment was repeated with identical results.

Donor H.C. was also sensitive to diphtheria toxoid. That treatment of cells with PPD caused *specific* release of the TF concerned with tuberculin sensitivity was shown by the fact that transfer of sensitivity to toxoid could only be effected by the PPD-treated cell residue.

No evidence could be obtained that PPD caused significant damage to any major fraction of the cells.

Positive Response to Chorionic Gonadotrophin in Klinefelter's Syndrome. ROBERT B. LEACH, WILLIAM O. MADDOCK,* C. ALVIN PAULSEN, JOHN LANMAN, and WARREN O. NELSON, Detroit, Mich., and Iowa City, Ia.

Chorionic gonadotrophin stimulates the Leydig cells of normal men to secrete increased amounts of estrogen and androgen. Urinary estrogen excretion and clinical evidence of increased androgen secretion were taken as criteria of response in four patients having Klinefelter's syndrome who received chorionic gonadotrophin (APL) three times weekly for two weeks to seven months. The diagnosis of Klinefelter's syndrome was based on high urinary gonadotrophins, buccal smears or skin biopsies showing a female genetic sex pattern, and testicular biopsies revealing hyalinized seminiferous tubules and clumped Leydig cells.

During treatment, each patient showed a definite response. Urinary estrogen excretion increased from low to normal levels in two moderately eunuchoidal patients, R.W. and C.B., age 18 and 51 years. The younger patient experienced a marked increase in number of erections, penile growth, and a striking change to normal adolescent personality. The older patient, who was treated for only two weeks, showed no clinical response. Subsequently, a response to large doses of testosterone was noted only after a month of treatment. Patient J.B., age 27, was a normal appearing male. Potency was normal, and was not affected by treatment. Urinary estrogen excretion, normal before treatment, increased four fold by the third week of treatment. Patient H.T., age 15, had undescended testes and moderate puberal development. During therapy, the gonads descended to the external ring, erections increased in frequency, and penile growth was noted. Urinary estrogens were not determined.

It is concluded that the Leydig cells in Klinefelter's syndrome remain responsive to gonadotrophin stimulation. The chorionic gonadotrophin therapeutic test is not valid for separating hypo- and hyper-gonadotrophic types of eunuchoidism.

Effects of Prednisone on Glucose Tolerance in Liver Disease. CARROLL M. LEEVY, Jersey City, N. J. (Introduced by Harold Jeghers).

Compound E and its analogues alter glucose tolerance in normal subjects and produce hyperglycemia and glycosuria in the presence of latent diabetes. The influence of hepatic disease on encountered responses was studied in twenty-five patients with liver disease and a normal fasting blood sugar level. Eight patients had acute viral hepatitis, five fatty liver, and twelve moderately advanced cirrhosis. After patients had been on a 300-gram carbohydrate intake for three to five days, oral glucose tolerance tests were performed using 1.75 grams of glucose per kilogram of body weight. Twenty-four to forty-eight hours later, this test was repeated after giving 30 milligrams of prednisone in two divided doses, one and six hours prior to ingestion of glucose.

Results of the prednisone-glucose tolerance test were correlated with hepatic functional reserve. Glucose tolerance curves without prednisone were normal in eight patients, flat in seven, and diabetic in ten. Addition of prednisone induced a diabetic response in four patients with normal curves, converted a flat curve to a normal pattern in three patients, and increased the height of each of the diabetic curves. After improvement in hepatic function, prednisone-glucose tolerance tests were normal in three patients in whom diabetic curves were induced by prednisone, and in three patients in whom flat curves were not altered by steroids. Prednisone produced a diabetic curve after all evidence of liver disease had disappeared in two patients who subsequently developed clinical diabetes.

Citric Acid Metabolism in Bone and Joint Disease. HENRY M. LEMON, JULIUS H. MUELLER, III, WILLIAM H. CHASEN, and JOSEPH M. LOONEY, Boston, Mass. (Introduced by Chester S. Keefer)

Citric acid tolerance tests have been performed in 6 healthy individuals, 36 patients with osteoarthritis, rheumatoid arthritis of peripheral joints, and ankylosing spondylitis, and 24 patients with inoperable cancer. The latter included carcinomas of the breast, bronchus, pulmonary alveolus, thymus, ovary, endometrium, leiomyosarcoma and multiple myeloma. Venous serum citrate was determined using Ettinger's modification of the pentobromoacetone method (J. Biol. Chem., 1952, 199, 531) after overnight fasting, 5, 10, 20, and 30 minutes following rapid I.V. injection of 0.5 gm. sodium citrate. A two-fold increase in the normal serum citrate concentration was thereby produced within 5 minutes, which, however, did not result in any significant change in serum calcium, phosphate, or glucose concentrations at 30 minutes.

The mean fasting citrate concentration of normals and arthritics was identical (27 to 29 gamma per ml. $\pm$ 2 to 7), but that of cancer patients was significantly elevated (43 ± 21; S.E. diff. $= 4.5$, $t = 3.25$). The serum citrate response of cancer patients usually remained significantly

elevated at all intervals throughout the test. In all groups serum citrate concentration after 30 minutes approached initial values, without variations in the rate of removal of citrate except in the cancer group, in whom slightly greater rates were noted.

Although bone contains the highest tissue concentration of citric acid (0.8 to 1.6 per cent), the fasting citrate level either may be elevated in the absence of roentgen evidence of bone destruction, or normal in patients with biopsied osseous metastases. Since all patients with elevated serum citrate levels also had increased copper resistant serum acid phosphatase (Reynolds *et al.*, Cancer Research, 1956, **16**, 943), it seems most likely that these observations reflect the metabolic activity of cancerous tissue which is relatively rich in citric acid compared to normal soft tissues.

The Mechanism of the Auricular Gallop. JAMES J. LEONARD, ARNOLD M. WEISSLER, and JAMES V. WARREN,* Durham, N. C.

The auricular (presystolic) gallop is a low frequency sound occurring after auricular systole and before subsequent ventricular contraction. With careful auscultation, this sound may be heard frequently in patients with hypertension, coronary artery disease, and first degree heart block.

Eighteen patients with readily audible auricular gallop rhythm were investigated. Blood pooling was effected by occlusive cuffs on all four extremities. Apex cardiograms and logarithmic phonocardiograms were recorded before, during and after inflation of these cuffs to diastolic levels for five minutes.

With cuff inflation, the gallop sound migrated towards the first heart sound, as did its equivalent wave on the apex cardiogram. Accompanying this movement was a striking diminution, or disappearance of the gallop in twelve cases. When the auricular vibrations did not completely disappear, they were seen as small, slow deflections blending with early components of the first heart sound. Upon release of cuffs, they moved back to their former position.

Auricular contraction produces a pressure wave in the ventricle, thought to cause tensing or even reclosure of the AV valve. The observations reported here suggest that under certain circumstances, transmission of this wave is accelerated. As a result, an auricular component of the first heart sound appears early and is split off from the remainder. If the separation is wide enough (>0.04 sec.), the auricular sound is heard separately, creating a gallop rhythm. The hemodynamic effect of blood pooling returns the auricular sound components to a more normal position. At times separation may be enhanced by a delay in the appearance of the major vibrations of the first sound, evidenced by slight prolongation of the Q-1 time. In patients with heart block and auricular gallop, similar separation of an auricular sound component occurs because of the conduction delay between auricle and ventricle.

The Genesis of Hypochloremia in Chronic Respiratory Acidosis. HOWARD LEVITIN, WILLIAM BRANSCOME, and FRANKLIN H. EPSTEIN,* New Haven, Conn.

Acute studies of CO_2 inhalation in man and animals have heretofore failed to demonstrate losses of chloride in the urine in excess of sodium. It has even been suggested by some that the characteristic hypochloremia of respiratory acidosis might arise from transfer of chloride to an intracellular position or from dilution of the extracellular fluid with sodium bicarbonate rather than from increased excretion of chloride by the kidneys.

Rats were placed in metabolism cages in an atmosphere containing varying concentrations of CO_2. One group was allowed food and water *ad lib.* during 2 to 3 days of exposure to 7½ per cent CO_2 or 12 per cent CO_2 in air. A second group, trained to take their 24-hour requirement of food in a 30-minute feeding interval at the beginning of each day, was studied during a control period of three days in room air and then placed in 5 per cent CO_2 or 10 per cent CO_2 for 24 hours. All rats were exsanguinated at the end of each experiment.

Breathing CO_2 produced an elevation in serum bicarbonate and a reciprocal depression of serum chloride of about 5 mEq. per L. with 5 per cent CO_2, 10 mEq. per L. with 7½ per cent CO_2, and 20 mEq. per L. with 12 per cent CO_2. These alterations were as marked at the end of one day as after three days. Renal losses of Cl^- were increased on the first day of hypercapnia to produce a negative balance of Cl^- at the end of 24 hours after which no further net loss occurred. Negative balances of K^+ and sometimes of Na^+ accompanied chloruresis in rats of group I but not of group II. Excretion of NH_4^+ increased on exposure to CO_2 and returned promptly to normal when the rats were given room air to breathe.

These experiments imply that renal compensation, characterized by the excretion of Cl^-, plays an important role in adjustments to respiratory acidosis in the rat. They further suggest that the chloruresis induced by breathing CO_2 may provide a convenient tool for the study of renal tubular mechanisms for the excretion and reabsorption of anions.

An Immunological Approach to Problems of Blood Coagulation. JESSICA H. LEWIS * and PAUL DIDISHEIM, Pittsburgh, Pa.

Following repeated injections of certain human plasma or serum fractions, rabbits developed high titer of antibodies, demonstrable by precipitin reactions. In addition the antisera developing in the injected rabbits specifically inhibited certain human coagulation factors but not the rabbits' own factors. A citrate eluate of barium sulfated normal serum was shown to contain high concentrations of the clotting factors PTC (plasma thromboplastin component) and proconvertin (Factor 7) and traces of prothrombin. After injection with this fraction rabbits developed antibody with marked anti-PTC and anti-proconvertin activity and moderate anti-prothrombin activity.

If a similar fraction was prepared from serum of a PTC deficient individual, the fraction contained proconvertin and traces of prothrombin, and after immunization with this fraction the rabbit anti serum showed no anti-PTC, but marked anti-proconvertin and a trace of anti-prothrombin. This anti-prothrombin, and probably other nonidentified inhibitors, could be removed by adsorption with plasma from a patient congenitally deficient in proconvertin (Stuart). After absorption the anti serum still showed marked clot inhibiting effects, prolonging normal plasma recalcification time, preventing normal thromboplastin generation and reducing proconvertin titer as tested by Owren's method or a two-stage method on isolated materials. Control uninjected rabbit plasma did not show any inhibitor or precipitin reaction with normal human plasma. The injected rabbits showed normal coagulation mechanisms.

The demonstration that antibodies to human PTC, proconvertin and prothrombin can be formed suggests that, in the future, precipitin reactions employing specific antibodies may be useful as methods for assaying human clotting factors.

Stimulation of Adrenocortical Secretions after Air Encephalography. AMOS H. LIEBERMAN and JOHN A. LUETSCHER, JR.,* San Francisco, Calif.

Aldosterone output appears to be dependent on alterations in the volume of a component of the extracellular fluid, while 17-hydroxycorticoid excretion is not affected by similar changes in hydration. To determine the effect of acute reduction in cerebrospinal fluid volume, seven patients have been studied before and on the day of air encephalography.

In four patients, no effort was made to control fluid or food intake. On the day of encephalography, there was a regular fall in urine volume and sodium content, while no consistent change in potassium excretion was seen. Aldosterone output increased two to three fold. Initial levels of 17-hydroxycorticoids of 4.7 to 7.1 mg. rose to 8.6 to 18.6 mg. per day. There was no consistent change in 17-ketosteroids.

In order to minimize dehydration and sodium depletion, oral intake was supplemented by intravenous fluids in three other patients, so that water, sodium, and potassium intake approximated control levels. On the day of encephalography, urine volume fell, and sodium output was unchanged. Potassium excretion rose in two cases. Aldosterone output increased in two cases. Elevation in 17-hydroxycorticoid excretion was similar to that observed in previous cases.

Acute reduction in volume of cerebrospinal fluid is followed by increased adrenocortical secretion of aldosterone and 17-hydroxycorticoids. These changes are not entirely dependent on dehydration or electrolyte depletion. Similar increases in aldosterone and 17-hydroxycorticoids have been noted after acute stimulation with exogenous corticotropin.

Quantitative Aspects of the Stimulus Resulting in Pain in Esophagus, Small and Large Intestines. MARTIN LIPKIN and MARVIN H. SLEISENGER, New York, N. Y. (Introduced by Robert F. Watson).

The relationship of stimulus intensity and duration required to produce pain in esophagus, ileum and colon has been studied. In each experimental determination a column of water was suddenly connected to a water-filled balloon-catheter system previously placed in the lumen of the organ. The height of the column of water was maintained constant during each determination. The magnitude of the pressure source and the time to onset of pain were measured. The experimental apparatus was identical in all determinations. Normal subjects were chosen for measurements of esophagus and colon, and subjects with ileostomy for measurements of ileum.

For each of the three organs it was found that as the intensity of the stimulus decreased, the duration required to produce pain increased. Values comprising the intensity-duration curves plotted for the three organs were found to be very similar. The variability of responses was greatest in the colon. Pressure declines in the tubing, and elastic properties of the balloons were measured, and it was determined that the intensity-duration curves would remain qualitatively unchanged if the stimulus intensity values were corrected to the actual pressure applied to the wall of the viscus.

The results of the study suggest that the rate of onset of pain is a function of the rate of distension of the viscus. To evaluate this possibility, an experimental procedure has been developed to measure the rate of distension and pressure applied directly to the wall of the viscus during distension.

Metabolic Studies of Sodium Depletion in Adrenalectomized Humans. MORTIMER B. LIPSETT and OLOF H. PEARSON,* New York, N. Y.

The detailed adjustments to salt depletion and subsequent repletion are difficult to study in man because of the efficient renal conservation of sodium. However, the continued renal salt loss, which occurs in the adrenalectomized human receiving a low salt diet and maintained with cortisone, permits observations of the phenomena accompanying sodium depletion.

Three adrenalectomized patients and one with Addison's disease have been followed with metabolic balance techniques through periods of low and high salt intakes to induce sodium depletion and repletion. Negative sodium balances of 600 mEq. were reached in two patients with initially normal total exchangeable sodiums before symptoms developed. The two other patients with initially low total exchangeable sodiums (21 and 23 mEq. per kilogram) developed hyponatremia and became moderately ill after net sodium losses of 135 and 180 mEq.

During the early stages of salt withdrawal, the negative sodium balance and decrease in weight suggested loss of extracellular fluid. As sodium depletion continued, the weight loss did not keep pace with the salt loss, implying some conservation of water in the face of con-

tinued sodium depletion. During this latter period, hyponatremia developed.

Although cortisone replacement remained constant, salt depletion was accompanied by negative nitrogen balances of 1 to 2 grams per day and creatinuria. This negative nitrogen balance was reversed and the creatinuria dropped sharply when salt was returned to the diet.

Positive sodium balances as high as 1,000 mEq. occurred with sodium repletion. Initially, however, there was little weight gain, although plasma volumes and chloride spaces expanded and the sodium entered the chloride space. This has been interpreted to mean that, although exogenous water was available, the first response to sodium replacement was a shift of intracellular water into the chloride space. These phenomena emphasize the complexity of the problems associated with "uncomplicated" salt loss.

Production of Carbon Monoxide by Hemin Oxidation. G. D. LUDWIG, W. S. BLAKEMORE, and D. L. DRABKIN, Philadelphia, Pa. (Introduced by F. C. Wood).

The production of CO by oxidation of hemoglobin and hemin has been demonstrated *in vitro*. These studies support Sjöstrand's thesis that CO is formed in man and other animals in the destruction of hemoglobin. More detailed investigation of the coupled oxidation of crystalline hemin (pyridine hemochromogen) and of various reducing substances (hydrazine, ascorbic acid) shows that CO is produced concomitantly with green pigment (verdohemochromogen) and results from fission of the ring at the α-methene bridge carbon. In different phases of these experiments CO has been measured by 1. Infrared gas analyzer; 2. Spectrophotometric recording of CO complexes of hemoglobin and of alkaline cytochrome c; Chemical methods dependent upon reaction with 3. Iodine pentoxide or 4. Palladium chloride; 5. Production of radioactive $BaCO_3$ following oxidation of CO formed from labeled hemin.

Crystalline hemin, containing 8 labeled C^{14} atoms per molecule (each methene bridge C and an adjacent C in each pyrrole), was oxidized by the Warburg-Negelein method; the resultant CO quantitatively converted to CO_2 and precipitated and counted as $BaCO_3$. The specific activity of the recovered radioactive CO_2 permitted the conclusion that the CO arose from one of the four methene bridge carbons (presumably the α-methene C), since no evidence of dipyrrole formation or further degradation of pyrroles was obtained and Lemberg has demonstrated that the bile pigment produced by this reaction is of the α-urobilin type.

Kinetic studies show that the peak of CO production lags far behind that of an intermediate verdohemochromogen, in which the α-methene C is oxidized to a carbonyl group, as claimed by Fischer and others.

Assuming that the same yield of CO per molecule of hemin decomposed is obtained *in vivo* as *in vitro*, then the amount of CO that is produced in the daily catabolism of hemoglobin is a major factor in the blood CO concentrations found in non-smoking healthy subjects.

A Pathophysiological Study of Myoclonus Due to a Virus. CHARLES N. LUTTRELL and FREDERICK B. BANG,* Baltimore, Md.

Myoclonus is a characteristic manifestation of Newcastle disease virus encephalomyelitis in chickens, mice, cats and monkeys. This study analyzes myoclonus and encephalomyelitis in experimentally infected cats.

Intracerebral, intracerebellar and intracervical cord innoculation of the virulent strain of Newcastle disease virus regularly produced encephalomyelitis in adult cats. In kittens, moreover, this was achieved by intranasal and intraocular routes. They, like adult cats, developed an encephalomyelitis with myoclonus.

Electroencephalograms and electromyograms were obtained serially in seven animals, and suggested the myoclonus originated from a deep lying subcortical focus or foci.

Serial sections of brains and cords of cats infected by the following routes: 5 intranasal, 4 intraocular, 5 intracervical cord, 2 intracerebral and 2 intracerebellar were examined and compared with two controls. The preponderant histological abnormalities were found in hypothalamus, brain stem and spinal gray. The type and distribution of lesions were similar to those of encephalitis lethargica. The histopathological similarities of these two encephalitides associated with the increasing variety of viruses belonging to the myxovirus group which cause encephalitis in both man and animals suggest the possibility that encephalitis lethargica was a member of this group.

A total of 12 brain stem and 8 high spinal transections were performed in cats with Newcastle disease virus encephalomyelitis and myoclonus. In 11/12 of brain stem and 7/8 of spinal animals the character and rate of the myoclonus were unchanged. In these cats it seems reasonable to attribute myoclonus to some "pathophysiological" change occurring at brain stem level or below. Moreover, it is clear that myoclonus in cats with Newcastle disease virus encephalomyelitis can occur at the organizational level of spinal cord, and that suprasegmental structures are not necessary for its development or maintenance.

The Uptake and Metabolism of S^{35} Labeled Thiourea, Thiouracil, and Potassium Thiocyanate by the Thyroid and Other Tissues. F. MALOOF and M. SOODAK, Boston, Mass. (Introduced by J. Lerman).

Thiourea, thiouracil and potassium thiocyanate (KSCN) have been utilized to study the metabolism of iodide by the thyroid for over a decade, yet the precise mode of action of these drugs remains unknown. Observations were made following the administration of these compounds, labeled with radioactive sulfur (S^{35}), to rats and following the incubation of these compounds with thyroid slices and thyroid homogenates.

These compounds reach their site of action in the thyroid within minutes following their administration to rats. Only a small fraction (0.5 to 2.0 per cent) of the administered dose is taken up by the thyroid. There is

no concentration gradient for these compounds in the thyroid versus serum. Furthermore, the observations reveal that their uptake by the thyroid is not under the influence of the pituitary and is not depressed by compounds which lower the uptake of I^{131} by the thyroid.

The experiments with rats demonstrate an interesting and unique degradation of these S^{35}-labeled compounds by the thyroid in contrast to the liver and serum. There appear to be several common denominators involved in the metabolism of S^{35}-thiourea and the iodination mechanism in the rat thyroid: (1) both are greatly diminished by hypophysectomy; (2) both are inhibited by the administration of Sulfadiazine, and (3) the amount of S^{35}-thiourea metabolized and the amount of thyroxine formed per day by the rat thyroid are of the same order of magnitude.

KSCN is known to displace iodide from the thyroid. The goitrogenic effect of KSCN can be overcome by iodides. The present data demonstrate that iodide does not displace KSCN from the thyroid, but rather inhibits its degradation in the thyroid.

These studies establish the minimal concentration of these compounds in the thyroid necessary to inhibit the concentration of I^{131} and the formation of protein bound I^{131} by the thyroid, approximately 2.6×10^{-5} M.

A Relationship Between Human Erythrocyte Aging In Vivo and the Activities of Glucose-6-Phosphate and 6-Phosphogluconic Dehydrogenases. PAUL A. MARKS, New York, N. Y. (Introduced by Alfred Gellhorn).

Erythrocytes derive most of their energy from glucose utilization. It is well established that the pentose phosphate pathway (PPP), in addition to anaerobic glycolysis, plays an important role in RBC metabolism. Little is known, however, about the changes occurring in carbohydrate enzyme levels associated with red blood cell (RBC) aging *in vivo*.

PPP activity in RBC was evaluated by applying to hemolysates specific assays for glucose-6-phosphate dehydrogenase (G-6-P.D.) and 6-phosphogluconic dehydrogenase (6-P.G.D.), which respectively catalyze the reactions: glucose-6-phosphate + triphosphopyridine nucleotide (TPN) $\rightleftharpoons$ 6-phosphogluconolactone + reduced TPN (TPNH) and 6-phosphogluconate + TPN $\rightleftharpoons$ ribulose-5-phosphate + CO_2 + TPNH.

Average dehydrogenase activities, expressed in units/10^9 RBC, in 30 normal subjects were: G.-6-P.D. 443 ± 108 and 6-P.G.D. 660 ± 128. Patients with reticulocytosis associated with various types of hemolytic anemia (6), hemorrhage (2), neoplastic disease (3), and therapy of iron deficiency anemia (3) and pernicious anemia (1), had significant elevations in activities of G.-6-P.D. (range 680 to 1160) and 6-P.G.D. (range 920 to 1420). In 13 diabetics not requiring insulin and 19 neoplastic disease subjects, the average enzyme activities did not differ significantly from normal.

Reticulocytes and young mature RBC compared to older RBC are less dense and more resistant to hypotonic hemolysis. Following centrifugation of both normal bloods (reticulocytes < 1.0 per cent) and hemolytic anemia blood (reticulocytes: 35 per cent) the upper RBC layer had higher enzyme activities than the lower RBC layer. Subjecting aliquots of normal bloods to graded hypotonicity revealed lower enzyme concentrations in the less osmotically resistant RBC.

These data strongly suggest that associated with maturation and aging of the RBC *in vivo*, there is an alteration in at least two enzymes involved in glucose metabolism. Increased levels of these enzymes may be a more sensitive indicator of young cell populations than reticulocyte counts. These enzymes catalyze reaction providing mechanisms for glucose oxidation with TPNH generation and pentose phosphate formation. A decrement in their activity may be an important aspect of RBC aging.

The Metabolic Effects of Glutamic Acid and Arginine in "Hepatic Coma" with Hyperammoniemia. WILLIAM V. MCDERMOTT, JR., DOROTHY H. HENNEMAN, and CLAUDE LAUMONT, Boston, Mass. (Introduced by R. D. Adams).

Recent conflicting reports on the value of L-glutamic acid in the treatment of "hepatic coma" have not clarified the biochemical effects of this amino-acid.

Ten patients with elevated levels of blood ammonia and with disturbances of the central nervous system were selected and the effects of parenteral sodium glutamate studied. Ammonia, glutamine, alpha keto-glutarate, phosphate, citrate, pyruvate and lactate were measured before, immediately after the infusion and at various times in the subsequent 48 hours. In all cases the administration of the glutamate infusion was attended by a precipitous fall in the blood ammonia level, by a rise in glutamine, and by lesser rise in alpha keto-glutarate. Any other biochemical changes observed were neither consistent nor of sufficient magnitude to permit interpretation. Subsequent to the infusion, the ammonia level rose slowly and concomitantly the glutamine and alpha keto-glutarate levels fell. These observations suggest that the administration of L-glutamic acid to patients with hyperammoniemia is attended by a temporary binding of the ammonia in the form of glutamine and a further reaction of glutamine to form alpha keto-glutarate. The fact that this reaction is reversible may explain the conflicting clinical reports on the value of this amino-acid.

The effects of infusions of arginine hydrochloride were quite different. No immediate changes occurred but within 12 to 24 hours a steady fall in blood ammonia levels was observed. Glutamine and alpha keto-glutarate were unchanged. Corresponding improvement in clinical and electroencephalographic abnormalities occurred. These changes were long-lasting and have persisted for several weeks without further treatment.

It would appear that both amino-acids could be used to advantage, in the treatment of ammonia intoxication, glutamic acid for its immediate binding effect on circulating ammonia and arginine for its slower but more permanent effect on the clearance of abnormal amounts of ammonia.

The Production and Catabolism of Cortisol in Experimental Endotoxin Shock. JAMES C. MELBY, RICHARD A. DEWALL, JAMES L. STOREY, and RICHARD H. EGDAHL, Minneapolis, Minn. (Introduced by Wesley W. Spink).

The shock induced in dogs by endotoxin from Gram-negative bacteria is characterized by elevated 17-hydroxycorticosteroid concentrations in the plasma and the gradual disappearance of cortisol from the plasma when given intravenously. Adrenal cortical secretory activity was assessed directly by the measurement of the minute output of 17-hydroxycorticosteroids in adrenal venous blood in dogs with adrenal vein cannulas. Prior to the injection of endotoxin the adrenal 17-hydroxycorticosteroid output rose from 0 to 1 microgram per minute to 10.7 micrograms per minute in response to corticotropin.

Following shock induced by endotoxin the output rose within two hours to an average maximum of 9 micrograms per minute and diminished thereafter. The adrenal response to endotoxin shock was abolished by hypophysectomy. Response to corticotropin had declined to an average maximum of 6.5 micrograms per minute four hours after endotoxin. There was a similar diminution of adrenal responsiveness to corticotropin following endotoxin in the hypophysectomized animal. Dogs given a constant infusion of corticotropin (0.2 unit per minute) averaged an output of 15.3 micrograms per minute of 17-hydroxycorticosteroids after three hours. Subsequent to the production of shock the adrenal output of 17-hydroxycorticosteroids fell progressively to an average of 4.4 micrograms per minute in the presence of high concentrations of corticotropin.

These findings indicate that the elevated plasma 17-hydroxycorticosteroid concentration in shock produced by endotoxin reflects both a diminished rate of steroid catabolism and markedly increased adrenal cortical secretory activity which progressively diminishes even in the presence of high concentrations of corticotropin.

The Effects of Heat and Pasteurization on Albumin Preparations in Particular Reference to Radioisotope Labeled Materials. WILLIAM R. MERCHANT, S. P. MASOUREDIS, and ERIC ELLENBOGEN, Pittsburgh, Pa. (Introduced by T. S. Danowski).

Since its primary development as a blood volume expander, albumin in its commercial form has been used for numerous physiological and isotopic studies. Little attention has been directed to the fact that for precautionary measures this material has been pasteurized at 60° C for ten hours in the presence of stabilizers. In most instances only an increase in turbidity or an electrophoretic homogeneous peak in dilute solution has been used as a criterion of homogeneity of the protein. Broad inferences have been drawn from the use of this material as to the biological behavior of "native albumin."

Marked changes were observed even electrophoretically in heating human or rabbit serum at 56° C or 60° C for varying periods of time, where the alteration was reflected in increased peaks in the positions of alpha 2 and beta globulins and in the albumin peak splitting into two components.

Preliminary light scattering studies on commercial albumin preparations would indicate the weight average molecular weight of the solute is in the neighborhood of 300,000, compared to a light scattering molecular weight of 75,000 for pure crystalline human serum albumin.

It was still possible that biologically this heated material would behave as the unheated. Fraction V of rabbit plasma was prepared by Cohn's method six, one part treated as a commercial preparation, the other simply diluted with physiological saline. Both were labeled with I^{131} and injected into rabbits. Marked differences in the biological half life were observed, with the heated material disappearing more rapidly from the plasma.

The above would suggest that insufficient attention has been directed to establishing biological and biophysical criteria for proteins administered for clinical studies.

Active Function of Renal Tubules During Acute Tubular Necrosis. W. H. MERONEY and M. E. RUBINI, Washington, D. C. (Introduced by Stanley M. Levenson).

The opinion is generally stated that during the oliguric phase of acute tubular necrosis the glomerular filtrate re-enters the extracellular fluid by passive back diffusion through discontinuous or inert tubular walls, and the small portion which escapes to the bladder resembles an ultrafiltrate of plasma. The present observations are inconsistent with this view and indicate that most of the urine derives from a few essentially normal tubules functioning under intense stimulation.

In ten patients with oliguria following hypotension, the osmolar U/P approximated unity. Urinary sodium concentration, always lower than plasma, fell progressively on successive days of oliguria. Urinary potassium concentration, always higher than plasma, increased as sodium decreased. The downward trend of urinary Na/K ratio was abruptly reversed by hemodialysis, but the ratio did not return to the high values present previously, when the plasma abnormalities were similar, until diuresis occurred.

Glycosuria did not appear, although some patients had induced hyperglycemia. In other oliguric patients extreme, sustained hyperglycemia was produced without the appearance of glycosuria. During diuresis hyperglycemia caused glycosuria.

Sodium conservation and potassium secretion, which were shown by dialysis to be partially independent of plasma concentrations, improved before urinary volume increased, suggesting an adaptive response of tubules already functioning rather than the contribution of newly healed nephrons. Inactive tubules could not have contributed significantly to the final urine without reducing the urine:plasma difference and increasing the urinary volume. The absence of glycosuria during hyperglycemia further demonstrates tubular activity and implies a severe reduction in glomerular filtration rate.

During diuresis the trickle of processed urine from the few active tubules is obscured by an overwhelming flood

of "ultrafiltrate" conducted by the most severely damaged tubules, which are newly patent but still unhealed.

Abnormalities of Blood Coagulation Produced in Rabbits by Feeding Cholesterol. CLARENCE MERSKEY, Capetown, South Africa. (Introduced by David Gitlin).

Twenty-seven adult male rabbits were fed an adequate diet for 96 days. Cholesterol (600 or 1,000 mg. daily) was fed to 18 of these, which produced coagulation defects as judged by prolongation of coagulation time (in 12) and prothrombin consumption defects (in 15). Plasma prothrombin and factor VII were markedly increased. Blood thromboplastin generation was defective, the abnormality lying in the serum factor, the alumina treated plasma being exceptionally active. Addition of normal or hemophilic serum corrected this serum defect, but serum from patients on phenindione therapy only partially corrected it whereas Christmas disease serum had no corrective effect. This suggested that the affected serum lacked Christmas factor, but this was not confirmed by direct mixing experiments. Affected rabbit blood added to Christmas disease or hemophilic blood shortened the coagulation time and corrected the prothrombin consumption defect of both these bloods if added in small proportion, but not in either if added in larger proportion. Blood or affected rabbits prolonged coagulation time and caused prothrombin consumption defects if mixed with normal blood. These findings suggested the presence of a circulating anticoagulant and possibly a defect of a third serum component (factor X?). It is possible that the mere presence of high concentrations of cholesterol (up to 2,000 mg. per cent) in the blood may cause the effects described, and preliminary studies in human hypercholesterolemic states lend some support to this concept.

Abnormal Binding of Vitamin B_{12} by Sera from Patients with Chronic Myelogenous Leukemia. A. MILLER, Boston, Mass. (Introduced by Charles P. Emerson).

Increased serum concentrations of vitamin B_{12} and prolonged disappearance times of intravenous cobalt^{60}-labelled B_{12} have been noted in chronic myelogenous leukemia. In the present study, sera from normal subjects and patients with blood dyscrasias have been tested for their vitamin B_{12} binding capacities by an equilibrium dialysis method and analyzed for their vitamin B_{12} concentrations microbiologically.

In 22 normal subjects, serum B_{12} concentrations ranged from 270 to 1,250 micromicrograms per milliliter and binding capacities from 630 to 2,185 micromicrograms per milliliter. In 11 patients with chronic myelogenous leukemia, serum B_{12} concentrations ranged from 4,200 to 14,000 micromicrograms per milliliter and binding capacities from 4,750 to 14,000 micromicrograms per milliliter. With treatment, binding capacities declined rapidly, reaching normal levels in two to three weeks, whereas serum B_{12} concentrations remained unchanged for several weeks and then slowly declined. Increased serum B_{12} concentrations and binding capacities were not observed in acute leukemia, chronic lymphocytic leukemia, polycythemia vera, and five of six patients with leukemoid reactions. One patient with chronic monocytic leukemia had levels similar to those in chronic myelogenous leukemia.

Sera to which excess Co^{60} B_{12} had been added were dialyzed to completion and then fractionated by paper electrophoresis. The β globulin contained the major fraction of the bound Co^{60} B_{12} in 6 of 7 normal sera whereas the α_1 globulin was the chief binding protein in 5 of 7 chronic myelogenous leukemic sera. The α_1 globulin contained 24 to 56 per cent of the bound B_{12} in patients with chronic myelogenous leukemia as compared to 3 to 9 per cent in normal subjects.

The increased serum B_{12} concentration and binding capacity in chronic myelogenous leukemia, associated with an increased binding of the labelled vitamin by the α_1 globulin, suggest a specific abnormality of serum proteins in this disease which is not generally found in leukemoid states and other blood dyscrasias.

A Study of Etiology of Macrocytic Anemia. O. NEAL MILLER and WALTER G. UNGLAUB, New Orleans, La. (Introduced by Grace A. Goldsmith).

Forty-six patients with macrocytic anemia have been studied with three procedures: 1) Schilling Test, 2) estimation of intrinsic factor (IF) in gastric juice and 3) estimation of serum factor. Methods for the last two procedures have been developed in this laboratory based on the previous observation that materials which contain intrinsic factor potentiate the ability of serum to combine with vitamin B_{12}. The patients were divided into groups according to their response to the Schilling Test. When absorption of radioactive B_{12} was stimulated by including IF concentrate with the radioactive test dose, the patient was classified as pernicious anemia (PA). Patients who showed normal absorption either with or without added IF were designated as nutritional macrocytic anemias (NMA). A third group absorbed little vitamin B_{12} with or without added IF and was classified as malabsorption syndrome (MAB). It was found that gastric juice from PA patients had only 25 to 30 per cent as much IF activity per ml. as normal gastric juice and similarly NMA and MAB patients had 70 per cent and 15 to 20 per cent, respectively. Serum factor for these three groups, as compared to pooled normal serum, averaged, 70 per cent for PA, 80 per cent for MAB and 100 per cent for NMA. Several patients having been diagnosed and treated for many years as PA were found to be MAB by these three procedures. Patients with MAB were lower in IF than patients with PA; both were low in serum factor. In NMA either serum factor or IF was low but not both. These data suggest that both intrinsic factor and serum factor are necessary to prevent all three types of macrocytic anemia.

The Relation of Maximal Oxygen Uptake to Cardiac Capacity and Arterial Oxygen Saturation in Normal Subjects and in Patients with Mitral Stenosis. JERE MITCHELL and CARLETON B. CHAPMAN,* Dallas, Tex.

Maximal oxygen uptake is thought to provide an index to cardiac pumping capacity in the absence of sig-

nificant pulmonary disease. Prior to testing this thesis, maximal oxygen uptake was determined in 73 normal men, aged 20 to 65, using a motor-driven treadmill. The value was found to decline with age. In 10 normal subjects, oxygen uptake and cardiac output (dye dilution method) were measured at increasing work loads. The mean maximal oxygen uptake was 3443 ± 430 cc. At this level, mean cardiac output had increased from 6.5 ± 1.7 L. per min. (resting, standing) to 26.1 ± 6.5 L. per min. At work loads greater than that producing maximal oxygen uptake, cardiac output tended to decline.

Determination of arterial oxygen content, PaO_2 (polarographic), oxygen capacity, and hematocrit during work showed no evidence of arterial desaturation or decline in PaO_2, even at loads producing maximal oxygen uptake. Simultaneous ear oximetry, however, regularly showed decline in saturation readings, apparently owing to factors unrelated to oxygenation of arterial blood.

In 7 patients with clinically significant mitral stenosis, maximal oxygen uptake was about half that found in normal subjects of comparable age. Simultaneous cardiac output measurements in 2 of the 7 showed very slight increases as work loads were raised. T-1824 appearance times tended to become longer with increase in work; in normal subjects, on the other hand, appearance time progressively shortened under the same conditions.

The studies support the thesis that maximal oxygen uptake is determined primarily by cardiac capacity in subjects without significant pulmonary disease. They also show that exercise of this type does not ordinarily produce arterial desaturation. Mitral stenosis lowers cardiac capacity and maximal oxygen uptake; the contours of T-1824 dye curves recorded during exercise in such patients come to resemble those seen in patients with cardiac failure as exercise loads are increased.

The Effect of Insulin, Alloxan Diabetes, and Phlorhidzin on Sugar Transport Across the Muscle Cell Membrane. H. E. MORGAN and C. R. PARK,* Nashville, Tenn.

The perfused isolated rat heart is a desirable test object for studying the transport of sugars through the muscle cell membrane. The preparation has no broken cells; sugars reach all cells rapidly due to the intact circulation and contraction. Viability is maintained for 1 hour or more without exogenous substrates.

Transport into the cell of the non-metabolized pentose, L-arabinose, can be determined by its intracellular accumulation. Intracellular sugar is estimated as the total tissue sugar minus the extracellular sugar. The extracellular space is determined simultaneously with the non-transportable sorbitol-1-C^{14}. Inward glucose transport is measured by disappearance from the perfusate.

Transport out of the cell is measured by perfusion of a heart containing intracellular L-arabinose with pentose free medium. After the initial 3 minutes, which removes 99 per cent of extracellular sugar, the arabinose appearing in the medium comes from inside the cells.

Glucose in the medium markedly inhibits the accumulation of intracellular L-arabinose; conversely, in high concentration L-arabinose inhibits glucose disappearance from the perfusate. Inward transport of each sugar is strongly inhibited by 3×10^{-3} M phlorhidzin and by alloxan diabetes. Transport is strongly accelerated by insulin.

The outward transport of L-arabinose is decreased by 48 hours fasting and alloxan diabetes. Insulin (3 μg. per ml.) causes a striking increase in outward transport approaching a maximum in about 5 minutes. The hormone effect is slower in the diabetic heart.

Both sugars share a reversible transport system distinct from diffusion and not involving phosphorylation. Transport is a site of the opposing hormonal actions of insulin and certain diabetogenic factors which thus regulate glucose supply in muscle. The metabolic action of phlorhidzin can be explained by transport inhibition.

Abnormal Cholesterol Metabolism in Lymphoma: A Comparison of Findings in Serum and Splenic Tissue. ELLY MOSCHIDES and MARIO STEFANINI,* Boston, Mass.

Abnormalities of cholesterol metabolism have been described previously in serum of patients with lymphoma (Am. J. M. Sc., 1956, 231, 155). They include: 1) low total cholesterol and cholesterol esters; 2) decrease of cholesterol migrating with the beta globulins fraction; 3) increase of cholesterol migrating with the albumin and alpha-1 globulins fractions. To elucidate the mechanism of these changes we have studied: a) content of total cholesterol and cholesterol esters; b) cholesterol esterases activity; c) distribution of cholesterol among the various protein electrophoretic fractions in the splenic tissue of 7 patients with lymphoma. These values have been compared to those obtained with serum. Splenic tissue was obtained surgically and immediately frozen in liquid N_2 after washing with cold saline. Autopsy or surgical spleens from patients with miscellaneous disorders represented a control group.

Findings in lymphoma: 1) Significant elevation of total cholesterol in splenic tissue as compared to a significant drop in serum. This was, however, a not specific finding which was noted in all pathologic spleens, irrespective of the disease entity involved. 2) Striking defect of esterification of cholesterol in both splenic tissue and serum of patients with lymphoma (and leukemias). Preliminary studies have indicated that such finding may be explained by decrease of cholesterol esterase activity in splenic tissue. 3) Relative increase of cholesterol-carrying beta globulins in splenic tissue. 4) Decreased cholesterol binding capacity of beta globulins in serum. It would then appear that defective enzymatic mechanisms and alterations in the tissues/serum balance of cholesterol-binding beta globulins might induce the striking alterations of cholesterol metabolism observed in patients with lymphoma.

These studies are among the first to indicate a definite metabolic abnormality in lymphoma; they stress the potential importance of biochemical studies of tissues in the interpretation of the pathogenesis of disease states.

Some Observations of the Effect on Serum Cholesterol of an Unsaturated Oil Administered Intravenously to Normal Healthy Adult Men. JOHN F. MUELLER,* JACK M. IACONO, and ALLAN L. FORBES, Denver, Colo.

The serum cholesterol-lowering potency of fed unsaturated fatty acids is not clearly understood. Intravenous fat emulsions provide an excellent tool to study this phenomenon, since the important effects of absorption can be by-passed.

Total, esterified and free cholesterols were measured in whole serum and its subnatant (spun at 12,000 rpm for 45 minutes) in 10 healthy young adult males of comparable ages before and at intervals after the intravenous injection of 90 gms. of a highly unsaturated oil. Six men received one injection separated by one week from oral ingestion of the same fat load. The other four received 5 daily infusions, cholesterol analyses being performed on the first, third, and fifth days.

In both groups there is a transient rise in whole serum cholesterol following the infusion which reaches its peak in four hours and then begins to fall. By 24 hours it is 18 per cent below the pre-infusion level. With oral administration there is no immediate rise and the fall is not so great, amounting to 11 per cent after 24 hours. In the four subjects receiving multiple infusions, the fall was progressive so that after 5 days the serum cholesterol was down 27 per cent.

The pattern for the subnatant or high density lipoprotein cholesterol was strikingly different. It fell precipitously during the infusion and continued to decline during the first 24 hours. Thereafter, each day the fall was less, so that on the fifth day the subnatant cholesterol actually rose. As with whole serum, the changes occurred primarily in the esterified fraction.

The rise in the whole serum cholesterol occurring simultaneously with a fall in subnatant at a time when the infused fat is rapidly disappearing from the blood suggests that endogenous cholesterol is utilized as a fat carrier or emulsifier, becomes associated with the low density or chylomicron lipoprotein component and disappears from the blood in this manner. Thus an overall decrease in serum cholesterol is attained.

The Hemodynamics of Mitral Valvular Disease. PAUL NOVACK, ROBERT C. SCHLANT, FLORENCE W. HAYNES, and ARTHUR O. PHINNEY, JR., Boston, Mass. (Introduced by Lewis Dexter).

Left heart catheterization was employed in the study of 41 patients with severe mitral disease. Aortic flow (AO) was estimated by the indicator dilution method, regurgitant flow (RF) by the Korner-Shillingford method, and mitral valve area (MVA) by the Gorlin formula. Patients were divided into three groups based on MVA: Group I, 15 patients, MVA $<$ 1.1 sq. cm. (predominant mitral stenosis); Group II, 11 patients, MVA 1.1 to 1.4 sq. cm. (mitral stenosis and insufficiency); Group III, 15 patients, MVA $>$ 1.5 sq. cm. (predominant mitral insufficiency). AO was greatly reduced in Groups I and II, averaging 2.1 (1.4 to 3.3) and 2.3 (1.2 to 3.1) L. per min. per M.^{2} and slightly reduced in Group III, averaging 3.1 (1.8 to 5.6) L. per min. per M.^{2}. The means and ranges of RF for each of the groups were: 0.91 (0 to 1.98), 1.35 (0.47 to 2.45), and 4.16 (1.50 to 7.89) L. per min. per M.^{2}, respectively. Total ventricular output (TF) was reduced in I, normal in II, and elevated in III: 2.8 (1.4 to 3.9), 3.6 (2.2 to 4.9), and 7.3 (3.4 to 13.5) L. per min. per M.^{2}, respectively. Significant mitral diastolic gradients occurred in all groups, averaging 23 (11 to 37), 14 (8 to 25), and 17 (9 to 27) mm. Hg. Left atrial mean pressures did not differ significantly among the three groups, averaging 26 (14 to 20), 23 (7 to 38), and 24 (13 to 35) mm. Hg. Marked elevations in left ventricular end-diastolic pressures were rarely encountered even where there was X-ray evidence of greatly enlarged left ventricles. These averaged 6 (−7 to 11), 8 (0 to 28), and 10 (1 to 15) mm. Hg. Central volumes, from left atrial injections, revealed a stepwise increase in the three groups, averaging 490 (230 to 804), 690 (410 to 1,100) and 970 (510 to 1,490) cc. These results suggest that pulmonary congestion in mitral insufficiency usually results from a high diastolic flow through a mildly stenotic valve rather than left ventricular failure.

Effect of Low Protein Diets Upon Serum Cholesterol in Man. ROBERT E. OLSON,* JOHN W. VESTER, DEHA GURSEY, and DORIS LONGMAN, Pittsburgh, Pa.

Kempner (Am. J. Med. 1948, 4, 545) observed a fall in serum cholesterol of hypertensive patients given the rice diet, and this has been confirmed by others. Although the hypocholesterolemic effect of the rice diet has been attributed to its low fat content, the relative contributions of other nutrients to this effect have not been previously evaluated. Since a marked hypocholesterolemic effect of alipotropic diets deficient in methionine and choline but high in fat has been noted in rats in our laboratory, it was decided to explore the effects of low protein moderate fat diets upon serum cholesterol in man.

Seven human subjects, five of whom were hypercholesterolemic, were fed a control diet containing 100 grams of protein, 80 to 100 grams of fat (mainly of animal origin), and 300 to 350 grams of carbohydrate for periods of 1 to 2 weeks. During the last three days of the fore period, the composition of the diet was rigidly controlled (P 100, F 80, C 320). The subjects were then fed a diet containing 25 grams of protein (mainly of vegetable origin), 80 grams of fat (50 from butterfat), and 395 grams of carbohydrate for one week and then returned to the control diet for four additional days. Fasting serum cholesterol was determined every other day. All subjects showed a decrease in serum cholesterol during the period of low-protein feeding, the range being -22 to -110 mg. per cent with an average of -52 mg. per cent despite sizeable intake of saturated fat, and all values returned toward normal upon resumption of the control diet. Changes in body weight were minimal during these relatively short term studies.

The results suggest that dietary protein may be one determinant of serum lipid concentrations in man and

are consistent with the epidemiological observation that populations ingesting relatively low amounts of protein have relative freedom from atherosclerosis.

The Effect of Potassium on Urinary Dilution. JACK ORLOFF,* DOUGLAS DAVIDSON, and HENRY N. WAGNER, JR., Bethesda, Md.

The initial step in the elaboration of hypotonic urine is thought to be reabsorption of sodium and attendant anion from isosmotic precursor urine in an area of limited permeability to water. Part and possibly all transport of sodium out of tubular fluid is effected by exchange for hydrogen and potassium ions. Secretion of hydrogen ions in exchange for sodium with a secondary mechanism for anion removal will decrease the concentration of osmotically active substances in urine, forming solute-free water (C_{H_2O}). On the other hand, secretion of potassium in exchange for sodium will not alter urine osmolality. Furthermore, the inhibitory effect of potassium on hydrogen secretion, and consequent decrease in sodium reabsorption, were this to occur at the diluting site, should interfere with the production of C_{H_2O}. To test this hypothesis the effect of intravenous potassium chloride on the excretion of C_{H_2O} in dogs was examined. Hypotonic saline was infused at a rate of approximately 1,000 micromoles per minute. When urine was maximally dilute, indicative of anti-diuretic hormone (ADH) suppression, potassium chloride was gradually substituted in equiosmolar amounts for sodium chloride. C_{H_2O} diminished in all studies in association with a rise in plasma potassium and an increase in potassium excretion. The fall in C_{H_2O} occurred despite an increase in inulin clearance, sodium excretion and total solute excretion. In contrast, enhanced potassium excretion produced by Diamox® did not effect a fall in C_{H_2O}. This may indicate a different site of action of this drug.

Although it is not possible at this time to exclude a stimulatory effect of the elevated plasma potassium on ADH secretion, the results are consistent with a direct renal effect of potassium. The enhanced secretion of potassium and the interference with hydrogen ion secretion may limit the extent of urinary dilution.

Personality, Behavior, and Vasopressor Mechanisms in Hypertension. ADRIAN M. OSTFELD and BENJAMIN F. LEBOVITS, Chicago, Ill. (Introduced by Mark H. Lepper).

This investigation was undertaken to ascertain any predictable features of personality, behavior, or vascular reactivity in essential hypertensive subjects. During periods of tranquility and of agitation, the least concentration of levarterenol in an isotonic phosphate buffer topically applied which caused constriction of bulbar conjunctival capillaries when observed by slit lamp examination was determined and called the capillary threshold. Brachial artery pressure responses to intravenous infusion of 20 gamma per minute levarterenol, injection of five units angiotonin and 400 mgm. TEAC were determined. Periods of agitation were associated with a statistically significant rise in supine systolic and diastolic pressures, fall in capillary threshold, and decrease in fall in pressure (and in seven instances a rise) during TEAC blockade. Such altered responses during agitation occurred in nine essential, one post-toxemia, and two renal hypertensives and four normotensives.

Twenty-five stress interviews were associated with significant diastolic pressure rises in seven of eleven essential, four of five renal, and one of two post-toxemic hypertensives, one subject with Cushing's syndrome, and three of six normotensive subjects.

The psychological Test Battery consisted of Wechsler-Bellevue Intelligence Test, Minnesota Multiphasic Personality Inventory, Bender Gestalt, Thematic Apperception Test, Rorschach, and a projective questionnaire. Twelve hypertensive demonstrated no predictable test score pattern.

The distribution of psychiatric, psychophysiological, and psychosomatic illnesses was determined in 20 hypertensives and in normotensives, similar in age, sex, race, social class, occupation, education, and post and current residence. No difference in qualitative or quantitative distribution of these illness types was discernible in the two groups.

In this study, no personality, behavior, psychological test performance, nor vascular response has been found that would distinguish a hypertensive subject.

The Effect of Morphine Upon the Renal Excretion of Water and Solute in Man. SOLOMON PAPPER, LAWRENCE SAXON, MAURICE B. BURG, HAROLD W. SEIFER, and JACK D. ROSENBAUM,* Boston, Mass.

It is commonly stated that morphine administration stimulates the release of antidiuretic hormone with resultant decrease in the rate of urine flow. Evidence on these points and information concerning the effect of therapeutic doses of morphine on glomerular filtration rate and solute excretion in normal man are limited.

An oral water load of 20 ml. per Kg. was established and maintained 20 times in 15 healthy subjects. During "maximal" diuresis, 7.5 to 30 mg. of morphine sulphate was administered subcutaneously, or 5 to 10 mg. intravenously. In 4 experiments the response was equivocal. In the remaining 16 studies, within less than 30 minutes urine flow decreased by 2.1 to 6.6 ml. per minute from control values of 12.1 to 22.2 ml. per minute, *unaccompanied* by a significant rise in urine osmolality. In 13 of the 16, the decreased diuresis was associated with a fall in endogenous creatinine clearance of 10 to 41 ml. per minute. In 14 there was a decrease in total solute excretion of 83 to 476 micro-osmols per minute.

In 7 of 20 experiments (in 5 of which nausea ensued) there was a later decrease in urine flow associated with a significant rise in urine osmolality 40 to 110 minutes after morphine administration, consistent with increased antidiuretic hormone activity.

These observations indicate that morphine, when administered in usual therapeutic doses to healthy men, may decrease the rate of water diuresis without evident re-

lease of antidiuretic hormone. Morphine does, however, induce a prompt decrease in solute excretion and a reduction in glomerular filtration rate, changes which may account for the diminution in diuresis.

Circulation and Respiration in the Giraffe. J. L. PATTERSON, JR.,* J. V. WARREN,* J. T. DOYLE, O. GAUER, T. KEEN, and R. H. GOETZ, Richmond, Va., Durham, N. C., Albany, N. Y., Bad Nauheim, Germany, and Capetown, South Africa.

The gravitational problems in the circulation of the giraffe, and the hardly less interesting respiratory problems created by the great tracheal length, have long intrigued physiologists. The present data were obtained on four animals in the Union of South Africa.

All of the animals were "hypertensive" by human standards, with arterial pressures at heart level of 282/158 to 344/194 mm. Hg, and 360/257 after bucking. Mean right atrial pressure approximated atmospheric. Pulmonary arterial pressure measured with the miniature manometer was 75/25 mm. Hg in one animal, and end-diastolic pressure in the left ventricle 5 to 7 mm. Hg. Cardiac output values (T-1824 or Fick) were 49, 41 and 19.4 L. per min. When one animal's head was lowered to ground level, arterial pressure at heart level showed little change.

Tracheal volumes in two autopsied animals were 1.2 and 2.3 liters. A pressure-volume diagram on the excised lungs showed the surprisingly small total lung volume of approximately 10 liters. Respiratory minute volumes ranged from 75 to 175 liters per min., tidal volumes from 2.7 to 4.1 liters and O_2 consumption from 2.7 to 4.7 L. per min. Respiratory rates were 36 to 58 per min. The inspiratory pneumotachogram in two animals was almost rectangular in shape. Both arterial O_2 and CO_2 tensions were normal or low normal by human standards, suggesting that in animals which were not hyperventilating the PO_2 values would be low. During 10 per cent O_2 inhalation, minute ventilation in the only anesthetized animal rose from 77 to 169 liters within 45 seconds.

These findings indicate that the giraffe solves the problem of cerebral perfusion by marked arterial "hypertension," doubtless aided by subatmospheric venous pressure at brain level. He probably compensates for large respiratory dead space either by large tidal and minute volumes or by adapting to some reduction in arterial PO_2. His airflow pattern is unusual, and tolerance to induced hypoxia poor.

Physiological Activities as a Function of Chemical Structure of Anabolic and Anti-anabolic Steroids in Man. MAURICE M. PECHET, Boston, Mass. (Introduced by Mark Altschule).

The physiological effects associated with alteration of chemical structure of steroids were investigated by complete metabolic balance studies on one normal, one panhypopituitary and three Addisonian subjects with the following 16 steroids: (1) prednisone (Meti-E); (2) prednisolone (Meti-F); (3) cortisone (E); (4) hydrocortisone (F); (5) 20β-dihydro-prednisolone (Meti-U); (6) 17-desoxyprednisolone (Meti-A); (7) 6-dehydroprednisone (Triene-E); (8) 4-chloro-prednisone (4cl-Meti-E); (9) 14α-hydroxy-hydrocortisone; (10) Δ^4-androstene-3,17-dione; (11) 11βOH-Δ^4-androstene-3, 17-dione; (12) 11βOH-$\Delta^{1,4}$-androstadiene-3,17-dione; (13) 1-dehydro-testosterone (Meti-testosterone); (14) 9αF, 11βOH-$\Delta^{1,4}$-androstadiene-3,7-dione; (15) 9αF, 11βOH, 17α-methyl-testosterone; (16) 1-dehydro-17α-methyl-testosterone (methyl meti-testosterone).

In the 21-carbon adrenocortical series 11β-hydroxylation accompanies anti-anabolic activities. These activities are enhanced by further chemical alteration such as 1,2-dehydrogenation and 9α-fluorination. 14α-Hydroxylation abolishes anti-anabolic activities. 4-Chlorination decreases anti-anabolic activities. 1,2-Dehydrogenation partially activates inactive C_{21} reduced adrenocortical steroid. In the 21-carbon series, 1,2-dehydrogenation is associated with: (1) decreased tubular reabsorption of sodium; (2) increased proportion of unconjugated 21-carbon metabolites; (3) markedly decreased conversion to 17-ketosteroids; (4) altering principal pathways of parent steroids' metabolism indicating cleavage of the dihydroxyacetone side chain preceding ring A reduction.

In the 19-carbon anabolic series, 1,2-dehydrogenation decreases anabolic activities. 11β-hydroxylation markedly decreases anabolic activities. 1,2-Dehydrogenation, 9α-fluorination and 11β-hydroxylation together abolish anabolic activities. 1,2-Dehydrogenation markedly decreases excretion of 17-ketosteroids, contrasting sharply with the metabolism of 19-carbon parent steroids.

An 11 oxygen atom is essential for anti-anabolic activities in the 21-carbon series. Enhancement of these activities by 1,2-dehydrogenation and 9α-fluorination depends on the presence of an 11 oxygen atom. The anabolic activities of 19-carbon steroids are markedly decreased by 11β-hydroxylation. Thus the presence or absence of an oxygen atom at C_{11} in 19-carbon and 21-carbon steroids largely determines the anabolic or anti-anabolic activities of these steroids.

The Influence of Arm Circulation on Clinical Circulation-Time Measurements. SOLBERT PERMUTT, MARIAN ISAACS, and EDVARDAS VARNAUSKAS, Baltimore, Md. (Introduced by Richard L. Riley).

When an indicator is injected in an antecubital vein and sampled in the contralateral brachial artery, the mean circulation time (C.T.) is significantly longer than when indicator is injected through a catheter in the subclavian vein. We consider this difference a measure of arm C.T. This difference is greatly diminished if the antecubital injection is made in an arm where blood flow is markedly increased by reactive hyperemia. Under these circumstances, arm C.T. is approximated by the difference in C.T. before and during hyperemia (Δ C.T.). To evaluate the influence of arm C.T. on total C.T. from elbow to tongue, we have measured Δ C.T. in 12 normal subjects and 14 patients with varying degrees of congestive heart failure. Three ml. of Decholin® were in-

jected in an antecubital vein, first, in the routine manner and then three seconds following the release of a blood pressure cuff, proximal to the needle, inflated above 250 mm. Hg for five minutes. In normal subjects, average total C.T. was 15.2 (S.D. 3.1) and Δ C.T. was 4.2 (S.D. 3.0). C.T. during hyperemia, which averaged 11.0 (S.D. 1.4), showed significantly less variation than total C.T. or Δ C.T., suggesting that variations in total C.T. of normal subjects are due chiefly to variations in arm C.T. In cardiac subjects, average total C.T. was 31.9 (S.D. 9.0) and Δ C.T. was 8.1 (S.D. 5.4), both values being significantly greater than in the normal subjects, suggesting that arm C.T. in cardiac patients tends to be prolonged in proportion to the increase in total C.T. The increased variability of total C.T. in cardiac patients appears to be due to variations in both arm and central C.T.

Studies in Adrenocortical Function in Liver Disease. RALPH E. PETERSON, Bethesda, Md. (Introduced by Harry Eagle).

Adrenal function in liver disease was studied by determination of rate of metabolism and rate of synthesis of hydrocortisone. Four patients with cirrhosis and one patient with acute viral hepatitis were studied. Patients with liver disease showed a 2 to 6-fold delay in rate of biotransformation of infused hydrocortisone. These same patients were able to metabolize the initial reduction product of hydrocortisone (dihydrocortisone) to tetrahydrohydrocortisone at the normal rate. They also showed no significant impairment in their ability to metabolize tetrahydrohydrocortisone to its glucuronide. The miscible pools and plasma hydrocortisone levels were normal or only slightly increased in the presence of low levels of urinary corticosteroids and 17-ketosteroids. The turnover rates of hydrocortisone were found to be decreased, 20 to 50 per cent of normal, when conventional tracer techniques, using hydrocortisone-4-C^{14}, were applied. These results are consistent with the interpretation that in liver disease there is a selective defect in the liver enzyme system (dihydrohydrocortisone dehydrogenase) necessary for the conversion of hydrocortisone to dihydrocortisone. This defect might be expected to cause an elevation of the plasma hydrocortisone level with a resultant suppression of ACTH production and a decreased secretion of hydrocortisone by the adrenal.

Serial Renal Biopsies in Toxemia of Pregnancy. VICTOR E. POLLAK, JOHN B. NETTLES, CONRAD L. PIRANI, ROBERT M. KARK,* and SJOERD L. BONTING, Chicago, Ill.

Nutritional deficiency, endocrine overactivity, and neurogenic stimuli from the gravid uterus are current theories of the etiology of pre-eclampsia and eclampsia. Percutaneous renal biopsies were made in 35 women diagnosed "toxemia of pregnancy." In 27, unique glomerular lesions were found; when serial biopsies were made after confinement, the lesions had disappeared. These characteristic pre-eclamptic lesions were readily distinguished from lesions in five cases of toxemia with essential hypertension, and from other types of renal disease in pregnancy (three cases).

In pre-eclampsia, blood corticoid and urinary aldosterone levels are elevated, but the renal lesions were unlike those we have seen in biopsies from patients with Cushing's syndrome, pharmacologic hypercortisonism, and primary hyperaldosteronism. Although pre-eclamptic women have abnormal pyridoxal metabolism, the renal lesions did not resemble those of experimental pyridoxine deficiency. To determine whether vasospastic agents capable of passing the placental barrier might cause the maternal renal lesion, the kidneys of ten fetuses born to pre-eclamptic mothers were examined; no abnormality was observed.

The characteristic lesion in pre-eclampsia is not a membranous glomerulonephritis as others have stated. In our material all glomeruli in the sections were affected in like degree, being larger than normal and almost completely bloodless. Details of the abnormalities became evident with the oil immersion lens. The observations were confirmed with periodic-acid-Schiff, Mallory-azan, van Gieson, reticulin, and fat stains. The glomerular basement membrane was thin and delicate; the swelling of the tuft was due to gross edema of the cytoplasm of endothelial and epithelial cells. Preliminary measurements of glomerular water (Lowry, J. Histochem., 1953, **1**, 420) confirmed that these glomeruli have an increased water content (0.105 Kg. dry weight per liter; normal 0.130 Kg. dry weight per liter).

These studies appear to support the concept that the renal lesion of pre-eclampsia develops as a result of neurogenic stimuli from the gravid uterus.

Relation of Filtered Bicarbonate to Bicarbonate Excretion During Diamox® Administration. RICHARD M. PORTWOOD, DONALD W. SELDIN,* FLOYD C. RECTOR, JR., and ROBERT CADE, Dallas, Tex.

The relationship between plasma [HCO_3^-] and HCO_3^- excretion during Diamox® administration was explored in normal subjects during maximum water diuresis in whom sharp reductions in plasma [HCO_3^-] and filtered HCO_3^- were produced by hyperventilation. During periods of comparable water, buffer, and HCO_3^- excretion, urine pCO_2-plasma pCO_2 was used as an approximate index of distal tubular hydrogen secretion.

Hyperventilation depressed plasma [HCO_3^-] from control values of 23 ± 1 to 16 ± 1 mEq. per L., with comparable falls in filtered HCO_3^-. HCO_3^- excretion rose slightly from 15 ± 9 to 29 ± 14 μEq. per min. Urine pCO_2 decreased from 43 ± 7 to 30 ± 7 mm. Hg. Urine pCO_2-plasma pCO_2 increased from 8 to 14 mm. Hg.

Diamox® plus hyperventilation depressed plasma [HCO_3^-] and filtered HCO_3^- to the same extent as hyperventilation alone, but markedly accelerated HCO_3^- excretion to 544 ± 150 μEq. per min. Urine pCO_2 was only slightly elevated to 50 ± 5 mm. Hg with a modest increase in urine pCO_2-plasma pCO_2 to 34 mm. Hg.

Diamox® alone did not depress plasma [HCO_3^-] or filtered HCO_3^- appreciably but augmented HCO_3^- excre-

tion to no greater extent than did hyperventilation plus Diamox®. However, urine pCO_2-plasma pCO_2 increased strikingly to 48 mm. Hg.

These data suggest that HCO_3^- diuresis produced by Diamox® is dependent, not only on filtered HCO_3^-, but also on the rate of H^+ secretion as influenced by blood pCO_2 and carbonic anhydrase activity. With hyperventilation alone, diminished CO_2 available for H^+ production will reduce H^+ secretion only modestly, since the carbonic anhydrase system is intact; HCO_3^- diuresis is therefore small. During hyperventilation plus Diamox® the low tissue pCO_2 during inhibition of carbonic anhydrase so impairs the uncatalyzed reaction that even small filtered loads cannot be reabsorbed, resulting in marked HCO_3^- diuresis of as much as 40 per cent of the filtered load. The diminished H^+ secretion under these circumstances is attested by the lower urine pCO_2-serum pCO_2 as compared with comparable HCO_3^- diuresis with Diamox® alone.

The Hypoglycemic Properties of Dimercaprol (BAL). THADDEUS E. PROUT, JOHN A. WEAVER, GEORGE W. SCOTT, and SAMUEL P. ASPER, JR.,* Baltimore, Md.

A study of the effect of dimercaprol (BAL) on carbohydrate metabolism was made in ten patients with diabetes mellitus and in laboratory animals. The observation of Butterfield (Lancet, 1955, 1, 489) that BAL reduces fasting blood sugar and glycosuria in diabetics was confirmed in two of seven diabetics with severe disease. In one severe diabetic, receiving constant diet and insulin, the fasting blood sugar decreased from 340 to 37 mg. per cent with symptoms of hypoglycemia on the third day of BAL administration. Insulin sensitivity was increased by BAL in three patients with moderate diabetes but was unaffected in three of those with severe disease. Irritation at sites of BAL injection limited clinical use.

BAL was administered intravenously to normal rabbits in increasing quantities, 0.06 to 0.36 mM per Kg. alone, or after prior mixing with insulin, 0.6 unit per Kg. The insulin effect was attenuated in a step-wise fashion with increasing quantities of BAL and abolished by BAL 0.18 mM per Kg. This latter quantity of BAL given alone, however, caused a 28 per cent decrease in blood sugar at three hours. In the normal rabbit BAL 0.18 mM per Kg. did not alter the rate of glucose disappearance following an intravenous glucose load, the rate of disappearance of labeled insulin from serum or the degree of insulin sensitivity. In adrenalectomized rats hypoglycemia following BAL was profound, and frequently fatal, but was diminished by cortisone. Increasing BAL to 0.36 mM per Kg., with or without insulin, produced hyperglycemia lasting more than three hours; the hyperglycemia appears to be an epinephrine effect since it was abolished by prior injection of ergotamine tartrate 1.0 mg. per Kg. in rabbits or by adrenal demedullation in rats.

The mechanism producing hypoglycemia is obscure. These studies demonstrate that BAL has a hypoglycemic effect in some diabetic patients and outline conditions which influence the response in laboratory animals.

Preparation of Growth Hormone from Human Pituitaries. M. S. RABEN,* Boston, Mass.

Human pituitaries were removed postmortem and stored in acetone; weeks to months later, the pituitaries were homogenized, washed further with acetone and dried in vacuum. Growth hormone was extracted and purified from the acetone-dried powder by the method originally devised for porcine pituitaries. The powder was extracted with glacial acetic acid at 70° C, an acetone precipitate discarded and the active fraction precipitated with ether. Corticotropin and intermedin were removed with oxycellulose, a precipitate at pH 8.5 was removed and growth hormone was brought down with the addition of alcohol. The yields from batches of 11.25 grams and 24 grams of powder were 350 mgs. and 1,100 mgs., or two and three times the usual yield from pig. As assayed in hypophysectomized rats, the human and porcine preparations were of similar activity, with the human perhaps slightly more potent. Evidence of anabolic effect in man was obtained in five patients in four different institutions by J. Beck and Venning, Pearson, Henneman, R. N. Beck and Raben and their respective co-workers. Growth hormone was also prepared by the same method from monkey pituitaries and found active in the one patient tested by Beck and Venning. The effective dose appears to be small enough (less than 0.1 mg. per kilogram) to make human autopsy pituitaries a practical source of growth hormone for clinical use, since preparations from slaughterhouse animal pituitaries have been generally ineffective in man. A fortunate feature of this method of preparation of growth hormone as applied to human pituitaries of indeterminate origin is the bactericidal and viricidal action provided by the use of acetone, ether and hot glacial acetic acid.

Kinetic Spectrophotometric Studies of a DPNH-Succinate Cytochrome c Reductase from Heart Muscle. MURRAY RABINOWITZ, RONALD ESTERBROOK, and BENEDETTO DEBERNARD, Philadelphia, Pa. and Madison, Wisc. (Introduced by William P. Chapman).

The synthesis of ATP, the source of energy for cardiac muscle contraction, is largely coupled to electron transport from substrate to oxygen. To gain further information about this process, the electron transfer system of beef heart muscle has been fractionated into simpler units and the function of these units examined. By cholate-$(NH_4)_2SO_4$ fractionation a particulate preparation has been obtained which contains as functioning components DPNH and succinic dehydrogenases, flavoprotein and cytochromes *b* and c_1; cytochrome oxidase and cytochrome *c* have been completely removed. Kinetic studies have been performed on this DPNH-succinate cytochrome *c* reductase using Chance's rapidly recording dual monochromator spectrophotometer.

It was demonstrated that the flavoproteins associated with DPNH and succinate dehydrogenases were different. Difference spectra of these two flavoproteins were individually recorded.

The velocity reduction of cytochrome c_1, a newly es-

tablished component of the electron transfer system, was several times greater than that of cytochrome *b*, but only one-third to one-fifth that of added excess cytochrome *c*. Cytochrome *c* addition failed to increase reduction velocities of cytochromes *b* or c_1. Flavoproteins were reduced at rates similar to that of added cytochrome *c*. These observations indicate direct flavoprotein interreaction with all three cytochromes in this preparation. The pattern of cytochrome reduction was identical with DPNH or succinate. The direct flavoprotein-cytochrome *c* pathway was inhibited by Antimycin A, in contrast to the Antimycin insensitivity of soluble DPNH cytochrome *c* reductase. Antimycin A decreased reduction velocities of cytochromes *c* and c_1, but markedly increased the reduction rate of cytochrome *b*. Amytal® inhibited the rates of reduction of all cytochromes by DPNH, but left unaffected flavoprotein or ferricyanide reduction. Succinate oxidation was unaffected.

Diffusion Characteristics of the Pulmonary Membrane and Capillary Bed in Various Diseases of the Lungs and Cardio-Vascular System. JOHN RANKIN, RONALD S. MCNEILL, and ROBERT E. FORSTER,* Philadelphia, Pa.

A method has recently been developed for calculating the diffusing capacity of the pulmonary membrane (D_M) and the average amount of hemoglobin in the pulmonary capillary bed (PCH) from a) the diffusing capacity of the lung for CO (D_L, which includes diffusion within the capillary as well as across the membrane) and b) the *in vitro* rate of combination of CO with intracorpuscular hemoglobin measured at several different alveolar O_2 tensions from 100 to 650 mm. Hg. If the hematocrit in the pulmonary capillaries is known, the pulmonary capillary blood volume (C_c) can be calculated from PCH. Measuring D_L at rest by a breath-holding technique, we have obtained D_M and PCH in 9 normal subjects and 20 patients with anemia, polycythemia, or cardio-pulmonary disease. The results are summarized as follows. In normal subjects D_M was about twice D_L, the latter averaging 32 ml. per min. per mm. Hg; V_c averaged 98 ml. on the basis of the peripheral hematocrit. In patients with diffuse pulmonary fibrosis, D_L was markedly reduced, owing in the main to a decrease in D_M, and to a lesser decrease in PCH in some instances. In patients with pulmonary vascular congestion as a result of heart disease, PCH was markedly increased, while D_M was normal or slightly decreased. In 4 out of 5 patients with anemia and no evidence of pulmonary disease, D_L was reduced and PCH was markedly reduced. In 2 of these patients D_M was also markedly reduced. These values tended towards normal as the anemia was corrected by therapy. PCH was increased in polycythemia.

Increased Susceptibility to Viral Infection Induced Experimentally by Psychophysiologic Stress. A. F. RASMUSSEN, JR.,* JAMES T. MARSH, and NORMAN Q. BRILL, Los Angeles, Calif.

A variety of factors other than specific immunity modifies host response to infectious agents, protecting against repeated exposure to potentially dangerous agents on one hand, and subtly converting innocuous exposures and inapparent infection into overt disease on the other. Psychologic stress is often proposed as one of the factors predisposing to overt infection. Patients with histories of repeated infections have shown increased resistance following psychotherapy. Clinical evidence supporting an etiologic role for emotional stress in recurrent herpes simplex is particularly impressive. As the initial step in an attempt at elucidation of psychologically induced changes in resistance, an experimental system demonstrating stress-induced increases in susceptibility of mice to infection with the virus of herpes simplex is described. The system follows techniques widely employed in experimental psychology for the production of behavioral changes in animals.

Avoidance-learning stress was produced by subjecting mice to repeated cycles of, first, warning stimuli, a bright light and buzzer, followed by electric shock, a painful stimulus. Animals were able to avoid the painful stimulus by jumping a barrier immediately after the warning stimuli and animals learned to avoid the shock fifty to eighty per cent of the time after two or three days. Restraint-stress was produced by confining mice in snugly wrapped wire mesh blankets. Mice subjected to either form of stress for periods of six hours daily for two to four weeks showed increased susceptibility to intraperitoneal inoculation with the virus of herpes simplex. The increase in susceptibility was manifested by statistically significant increases in mortality in stressed as compared to control animals, and by a trend toward shorter incubation periods in stressed animals.

An Hepatic Mechanism for Tolbutamide Hypoglycemia in Animal and Human Subjects. LILLIAN RECANT* and GEORGE L. FISCHER, St. Louis, Mo.

Hypoglycemia may result from decreased hepatic release of glucose, increased peripheral utilization of glucose, or a combination of the two processes. In an effort to define the mechanisms involved in tolbutamide-induced hypoglycemia, the following experimental observations were made.

Determinations of liver slice glucose release and diaphragm muscle glucose uptake were made on isolated liver and muscle removed from rats, at 15, 30, 45 and 60 minutes following I.P. tolbutamide. The time-curve of blood sugar fall correlated directly with a decrease in liver slice glucose release. No evidence of enhanced glucose uptake in muscle was found.

Human subjects were studied, utilizing the technique of hepatic venous catheterization. Measurements of peripheral arterio-venous sugars were also made. Following I.V. tolbutamide, a fall in hepatic glucose release associated with a 20 to 60 per cent increase in hepatic vein pyruvate was observed. No increase in peripheral glucose utilization could be demonstrated.

These findings suggested that hypoglycemia resulted from a retention of carbohydrate by liver with an as-

sociated increase in hepatic glucose utilization, as evidenced by the rise in pyruvate. The following study further supports this hypothesis. *In vitro* C^{14}-glycine incorporation into liver slice protein was studied in 24 starved control rats and in 23 starved rats pretreated with tolbutamide. The latter group showed a 54 per cent increase in incorporation rate. (Specific Activity increased from 65.5 to 101.3 counts per min. per 100 γ protein.) It has been shown previously that C^{14}-glycine incorporation depends upon active glycolysis and is known to be impaired in starvation and diabetes and to be restored by glucose and insulin.

The observations made are consistent with an hepatic insulin-like action following tolbutamide. Preliminary studies indicate that tolbutamide increases "insulin activity" in dog pancreatic blood. The absence of peripheral "insulin activity" following tolbutamide requires explanation and will be discussed.

Effect of Dialysis and Purine Ribosides Upon the Anemia of Uremia. SEARLE B. REES, WALTER G. SCHEITLIN, JOHN C. POND, THOMAS J. MCMANUS, WARREN R. GUILD, and JOHN P. MERRILL,* Boston, Mass.

Erythrocytes from uremic patients live a normal life span in healthy recipients despite increased random cell destruction in their host. We are, therefore, investigating remediable cellular and extracellular factors contributing to the anemia of uremia. Ten chronically ill uremic patients manifesting varying degrees of renal decompensation were selected for hemodialysis. All demonstrated a normochromic, normocytic anemia, hyperosmolarity, compensated metabolic acidosis, azotemia, electrolyte imbalance, and an anion deficit.

Pre-dialysis reticulocyte counts and Cr^{51} cell volume and survival studies revealed increased red cell destruction and inadequate erythropoiesis to compensate for this deficit. *In vitro* erythrocyte equilibrations revealed inability to maintain normal phosphate and potassium balance. Net resynthesis of phosphate esters and steady state potassium flux could be maintained when uremic cells were equilibrated in compatible fresh plasma fortified with a purine riboside, inosine. Uremic cells metabolize purine riboside via phosphorolysis and extra-glycolytic pathways as effectively as normal human erythrocytes.

All patients showed clinical improvement following dialyses calculated not to lower the blood urea nitrogen but which removed anion deficits. Although clinical improvement occurred in 2 to 3 days, red cell survival and *in vitro* equilibration characteristics did not return to nearly normal until 4 to 6 days after dialysis.

Administration of the purine riboside, inosine, partially reversed a biochemical lesion observed in uremic erythrocytes *in vitro*. These studies have been extended *in vivo*. Two patients received inosine intravenously. Within one hour the erythrocytes demonstrated normal potassium and phosphate ester contents.

Electrolyte Balance and Acid-Base Metabolism in Primary Aldosteronism. ARNOLD S. RELMAN* and WILLIAM B. SCHWARTZ,* Boston, Mass.

In two patients with severe primary aldosteronism, studies were carried out to define the nature of the disturbances in electrolyte and acid-base physiology.

1. As might be anticipated from the known influence of sodium intake on the kaliuretic action of mineralocorticoids, a significant negative balance of potassium occurred only when sodium was ingested. On a normal-potassium, low-sodium intake, potassium balance was slightly positive. Although urine concentrations were sometimes 5 to 6 mEq. per L. (daily volume 5 to 6 liters), potassium excretion was always inappropriately high compared to the low plasma levels. With potassium loading, one patient continuously retained a portion of the load until the 1,000-mEq. deficit was corrected. In the other patient, even on a low-sodium diet containing 250 mEq. of potassium, there was only a transient period of retention.

2. The urine was always relatively alkaline, and four-hour collections through the day showed little or none of the usual diurnal fluctuations in pH or acid excretion. With widely varied urine flows, bicarbonate excretion was nearly a direct function of flow. Ammonium and potassium concentrations varied widely, but concordantly, independently of pH and roughly inversely with flow. There was no inherent defect in the acidifying mechanism, however, since urine pH's as low as 5.1 to 5.2 occurred during sulfate loading.

3. There was no "paradoxical" response to cortisol or DOCA.

4. Correction of alkalosis at the beginning of the potassium-loading period was associated with a further rise in urine pH and a diuresis of bicarbonate, large enough in one case to explain the drop in serum bicarbonate.

5. In one patient, following removal of the adrenal tumor, a syndrome of hypoaldosteronism appeared, with persistance of defective sodium conservation and hyperkalemia for several months.

Physiological Implications of Thyroxine-Binding by Serum Proteins. JACOB ROBBINS and J. E. RALL,* Bethesda, Md.

Thyroxine in human serum is reversibly bound to an alpha globulin (TBP) and, in lesser degree, to albumin. Free (unbound) thyroxine is not directly measurable by present techniques. Utilizing thyroxine-I^{131} and reverse flow zone electrophoresis, and data in the literature for bovine serum albumin, one may calculate the association constant for thyroxine and TBP (8×10^9) and the concentration of free thyroxine in serum (mean normal, 6×10^{-11}M). These values, although approximate, appear valid for intercomparisons.

Free thyroxine was calculated in two groups of patients with paradoxical protein-bound iodine (PBI) levels. In euthyroid pregnant women, elevated PBI was

accompanied by an increase in concentration of TBP. The resulting free thyroxine was normal (3.5 to 6.0×10^{-11}M). Nephrotic subjects with low PBI had low TBP levels and, in all but one, free thyroxine was normal or elevated (3.1 to 32×10^{-11}M). In contrast, hypothyroid subjects had an elevated TBP, so that free thyroxine was relatively lower than PBI. In hyperthyroidism, TBP was unchanged; the increase in PBI was, therefore, accompanied by a relatively greater increase in free thyroxine (21 to 71×10^{-11}M). Thus, free thyroxine was a satisfactory index of clinical thyroid status in hypothyroidism and hyperthyroidism. In pregnancy and nephrosis, it appeared to be a better index than PBI.

To test whether the metabolism of thyroxine might also be related to free thyroxine, data from Berson and Yalow were used. Free serum thyroxine was calculated from the PBI and compared with thyroxine degradation. A linear relationship between these two variables was found.

It appears probable, therefore, that thyroxine bound to serum protein is inactive and that the level of free thyroxine in serum is one of the factors which govern the general metabolic effects of thyroxine and its rate of degradation.

The Nature of the Respiratory Acidosis of Sleep and of the Respiratory Alkalosis of Hepatic Coma. EUGENE D. ROBIN, ROBERT D. WHALEY, CHARLES H. CRUMP, and DAVID M. TRAVIS, Boston, Mass. (Introduced by Eugene C. Eppinger).

Carbon dioxide retention is known to accompany sleep, but the magnitude and mechanism of this retention have not been determined. A study of this phenomenon was, therefore, undertaken in fourteen normal subjects, in four patients with chronic hypercapnia and in five patients with hepatic coma.

Normal subjects during sleep show a rise in alveolar carbon dioxide tension to as high as 52 mm. Hg. There is a fall in alveolar ventilation, oxygen consumption and carbon dioxide production. Alterations in respiratory rhythm, including cyclic breathing of the Cheyne-Stokes variety, were observed in six of the fourteen normal subjects. The ventilatory response to carbon dioxide is depressed during sleep, falling from 1.3 to 0.3 liter per minute per mm. Hg. Onset and reversal of this depressed response occur almost simultaneously with sleep and wakefulness, respectively.

Chronic hypercapnia is associated with depression of the ventilatory response to carbon dioxide in a waking patient. This depression becomes more profound during sleep and this results in rises of alveolar carbon dioxide tension to as high as 70 mm. Hg. Cheyne-Stokes breathing during sleep was seen in three of four patients with chronic hypercapnia.

Hepatic coma is associated with alveolar hyperventilation and hypocapnia which are independent of the patient's state of consciousness. Even in the presence of this increased ventilation the respiratory sensitivity to carbon dioxide is not increased; indeed, in deep coma it is depressed. Thus the respiratory alkalosis of hepatic coma appears to result from the stimulation of the respiratory control system by factors acting independently of the carbon dioxide-pH system.

These data demonstrate that carbon dioxide retention in sleep is related to a depression of respiratory center sensitivity. The rapid development of this depression suggests that it is mediated neurogenically.

Pulmonary Vascular Responses to Serotonin and the Effects of Serotonin Antagonists. JOHN C. ROSE, Washington, D. C. (Introduced by Edward D. Freis).

In intact animals, 5-hydroxytryptamine (serotonin) elevates pulmonary vascular resistance. Pulmonary hemodynamics are influenced by extrapulmonary factors (right ventricular output, left atrial pressure, blood volume redistribution) and altered ventilation. The following experiments showed that the serotonin effect is due to direct pulmonary vasoconstriction.

A diaphragm pump of controlled output was substituted for the left ventricle in 16 dogs. Blood was drained from the left auricle to a reservoir, from which it was pumped to a T-tube in the thoracic aorta. Left ventricular bypass was complete. The right ventricle continued functioning normally.

As serotonin (2 to 40 mcg. per Kg.) was injected into the pulmonary artery, the tube draining the left auricle was temporarily diverted to a second reservoir. During this period, the circulation was maintained by blood pumped from the previously-filled main reservoir. The drug traversed only the lungs. When the main pump reservoir was nearly depleted, the auricular tube was returned to its original position and the blood in the temporary container was returned to the circulation.

Confined to the pulmonary vasculature, serotonin caused immediate pulmonary hypertension and decreased blood flow. Precisely similar pressure and flow changes followed the same dose when given at 5-minute intervals. No substance studied except serotonin produced pulmonary vascular effects in doses insufficient to affect systemic arterial pressure.

Systemic vascular reflexes were not initiated by serotonin in the lungs. (These reflexes are easily demonstrated in this preparation using veratrum.)

Reproducibility of pulmonary vascular response permitted evaluation of several "antiserotonin" substances. Phenothiazine (Phenergan®), 0.6 to 1.0 mg. per Kg., completely abolished pulmonary vascular response to serotonin. No other antihistamine studied had serotonin-blocking properties. Antiserotonin effect of phenothiazine was more potent than that of the serotonin anti-metabolite, BAS. LSD-25 and its 2-bromo derivative abolished pulmonary vascular effects of serotonin in slightly smaller doses than phenothiazine.

Zone Electrophoretic Studies of Serum Alkaline Phosphatase Activity. I. N. ROSENBERG,* Boston, Mass.

Samples of serum of abnormally high alkaline phosphatase activity obtained from patients suffering from

Paget's disease and from obstructive and infiltrative disease of the liver and bile ducts were subjected to electrophoresis in starch blocks. Sodium barbital buffer, pH 8.6, ionic strength 0.1 was used, and electrophoresis was conducted at 5° C for 48 hours at a potential difference of approximately 6 volts per centimeter. After electrophoresis, the block was divided transversely in 5-millimeter sections and the protein concentration and alkaline phosphatase activity of the filtered aqueous eluates of these sections were determined. Hydrolysis of p-nitrophenylphosphate, pH 10.3, was used to measure phosphatase activity.

In Paget's disease (seven cases) the serum electrophoretic pattern indicated nearly all the alkaline phosphatase activity to be in a single band whose peak was at, or slightly behind, the alpha-2 globulin peak; less than 5 per cent of the activity was in the alpha-1 globulin zone. By contrast, in 13 cases of obstructive jaundice and hepatic cancer, the serum phosphatase pattern showed at least two bands; the larger corresponded to the band observed in the Paget's cases, while the smaller, representing 6 to 19 per cent of the total activity, was of greater mobility, with a peak in the alpha-1 globulin region. A broad band of phosphatase activity with mobility approximately that of beta-globulin was noted in two cases of biliary cirrhosis, and a poorly-resolved zone of activity in this region was noted in several other cases of hepatic disease. The electrophoretic serum alkaline phosphatase patterns of four normal subjects qualitatively resembled those of Paget's disease. The results suggest that there may be qualitative differences in the patterns of the serum alkaline phosphatase activity in various clinical states.

The Use of Albumin-Heparin Infusion as an Anti-Lipemic Agent. RAY H. ROSENMAN,* San Francisco, Calif.

Lipemia clearing is preceded by hydrolysis of triglycerides, a reaction catalyzed by lipoprotein lipase and controlled by albumin since released fatty acids are complexed with albumin. Since such hydrolysis appears requisite for plasma clearance of triglycerides, the following studies were made of the possible use of albumin-heparin infusion as an anti-lipemic agent.

Hyperlipemia was produced in rats by inducing nephrosis, by Triton injection, and by injection of hyperlipemic rabbit serum. The rats were variously infused with serum albumin containing heparin, or with only albumin, heparin, or saline. The albumin-heparin infusion induced a marked fall of plasma triglycerides, phospholipids, and cholesterol in the nephrotic and Tritonized rats, and accelerated clearance of excess plasma lipids in those injected with lipemic rabbit serum, in each instance exceeding the response induced by separate injections of heparin or albumin. The differences weren't ascribable to variations of induced hemodilution. The amount of cleared lipids was proportional to both the magnitude of the hyperlipemia and to the amount of injected albumin, suggesting that the lipid-clearing mechanism obeys mass action principles.

Although the heparin-activated reaction appears fundamentally concerned with hydrolysis and clearance of plasma triglycerides, it is possible that it also is *directly* concerned with clearance of *excess* phospholipids and cholesterol from the plasma. Thus it was found that albumin-heparin injection also accelerated clearance of excess phospholipid and cholesterol from plasma of rats injected with such lipids contained in serum of bile duct-ligated rats, which is not turbid and contains no excess triglycerides. In contrast, no fall of plasma lipids occurred in *normal*, fasting rats infused with albumin-heparin.

Thus albumin-heparin infusion is an effective anti-lipemic agent and heparin-activated mechanisms may be concerned with phospholipid and cholesterol as well as triglycerides.

Observations on the Pathogenesis of Juvenile Pernicious Anemia. CYRUS E. RUBIN, Seattle, Wash. (Introduced by Wade Volwiler).

This is a pathogenetic study of eight well-authenticated cases of juvenile pernicious anemia, in whom the stomachs uniquely secrete HCl without producing intrinsic factor. This contrasts with the invariable achylia of adult p.a., a finding which has led to the presumption that gastric fundal atrophy causes defective intrinsic factor production.

The data suggest that juvenile p.a. constitutes the homozygous state for adult Addisonian p.a. Six patients had siblings with the same disease; three had parental consanguinity and others had a suggestive family history of adult p.a.

The ages ranged from 3.6 to 24 years in these eight patients. Seven patients now secrete HCl without stimulation although many were previously shown to have histamine-fast achlorhydria during repeated exacerbations. Treatment began before two years of age in six patients. The only patient with permanent achylia was initially treated at 12 years, the latest institution of therapy in this series. Considering the intrinsic factor deficiency in the acid secretors, the finding in one-third of them of pepsin levels in the achylic range was of interest.

Histologically, suction biopsies revealed ample parietal and chief cells in the seven acid secretors, unlike the extreme glandular atrophy observed in the child last treated and in all adult p.a. Minimal residual abnormalities possibly reflected gastritis during past exacerbations. The one patient biopsied during an exacerbation had hypochlorhydria and moderate gastritis. Large gastric columnar cells were exfoliated twice: in the achylic and in a patient with a previous, severe, protracted exacerbation.

Intestinal vitamin B_{12} malabsorption in addition to intrinsic factor deficiency was present in four patients.

These studies of earliest p.a. make it apparent that the essential hereditary feature is diminished production of intrinsic factor. Protracted subclinical vitamin B_{12} deficiency may then produce secondary irreversible atrophic

gastritis with its potentialities for polyps and carcinomas as frequently seen in adults.

Effect of the Type of Urinary Solute on the Process of Urine Concentration. M. E. RUBINI, W. B. BLYTHE, and W. H. MERONEY, Washington, D. C. (Introduced by Laurence H. Kyle).

The maximal osmolar concentration of urine attainable with dehydration and exogenous ADH is significantly increased following brief high-protein feeding. This effect of diet is independent of the total quantity of solutes ingested, and the degree of antecedent dehydration. Possible causes for the increased concentrating ability include adaptive augmentation of the tubular capacity to absorb water, or some property peculiar to the type of solute excreted.

Experiments were designed to exclude adaptation as a principal factor, and to test the hypothesis that urea may allow greater water reabsorption than other urinary solutes. Acute studies were performed in patients with hyposthenuria, normal subjects, and dogs, in which the contribution of urea to total urinary solute was abruptly increased.

Urea was given to patients after water deprivation and repeated injections of Pitressin®, and to hydropenic subjects and dogs during mannitol or sodium sulfate diuresis. Shortly after urea was given solute concentration and excretion rate increased. The $U_{Osm.}/P_{Osm.}$ rose or was unchanged. During osmotic diuresis, distal water reabsorption (T^cH_2O) was constant over a wide range of urine flow, irrespective of antecedent diet, but increased by 20 to 35 per cent when urea was added.

It would appear that the type of urinary solute affects the capacity for water reabsorption. Possibly urea, by polymerization, by aggregation with other urinary solutes, or by partial reabsorption, is not completely effective in the determination of a limiting osmotic gradient at the site of urine concentration. Tubular secretion of urea at some more distal site cannot be excluded. In each circumstance, urea would offer less resistance to water reabsorption than is implied by its molar concentration in the urine. Thus the increased urine concentrating ability induced by dietary protein may be due solely to augmented urea excretion.

Results of Clinical Application of Certain Microbiologic and Metabolic Observations in the Chemotherapy of Pulmonary Tuberculosis. W. F. RUSSELL, JR., S. H. DRESSLER, and G. MIDDLEBROOK,* Denver, Colo.

According to one microbiologic theory it should be possible to treat effectively with some combination of two sterilizing antimicrobial agents even the most advanced cases of tuberculosis, *i.e.*, without emergence of drug-resistant mutants and with bacteriologic sterilization of all "open," active, tuberculous lesions. For achieving this result, two specific conditions were deemed essential: 1) The metabolic disposition, hence the dosage, of these agents must be considered in relation to the problem of delivering a sufficient concentration of each to all bacilli in active lesions; and 2) The microbial population excreted must be susceptible to both agents.

To test this hypothesis, 113 consecutive patients excreting populations of tubercle bacilli susceptible to both isoniazid and streptomycin were observed for at least 6 months under treatment with daily high dosages of these drugs (streptomycin for at least 90 days). Additional pharmacologic agents were administered to certain patients, but in no instance were "physical" ancillary measures employed, such as strict bed rest, pneumotherapy, or thoracic surgery, earlier than 4 months after bacteriologic conversion. Within 6 months all patients, including 46 subjects with advanced cavitary, bilateral disease, became bacteriologically negative by all criteria; and no drug-resistant mutants had emerged.

Among a control group of 104 patients consecutively admitted to hospital during the same period, who were excreting tubercle bacilli resistant to either or both drugs as a consequence of previous treatment, and who were treated with these and other antimicrobial agents in equally high or higher dosages, plus ancillary physical measures, only 21 achieved bacteriologic conversion: 5 converted coincident with surgical intervention and 12 were excreting populations susceptible to one of these two drugs. Only 4 of these 21 were excreting populations resistant to both drugs on admission to this study.

These results confirm the microbiologic theory.

Whole Blood Transaminase Levels in Anemia. MARTIN SASS and PAUL W. SPEAR, Brooklyn, N. Y. (Introduced by Janet Watson).

In a study on the glutamic-oxaloacetic transaminase (G.O.T.) level of whole blood, three groups of patients were investigated. These consisted of 23 patients with no hematological abnormalities (mean hemoglobin 13.2 gm. per cent), 18 patients with mild to severe nonhemolytic anemia (mean hemoglobin 8.7 gm. per cent) and 18 patients with mild to severe hemolytic anemias, both congenital and acquired (mean hemoglobin 8.8 per cent). The mean G.O.T. level for the first two groups was 560 and 610 units per 100 mg. hemoglobin, respectively. The mean G.O.T. level for the hemolytic anemia group was 1,310 units per 100 mg. hemoglobin.

Two patients with acquired hemolytic anemia and two with megaloblastic anemia were followed during therapy. Elevated G.O.T. levels (1,750 and 1,650 units) were obtained for the former at the start of steroid therapy. Marked reticulocytosis and severe anemia were also present. Subsequent G.O.T. levels remained abnormally high (1,350 and 1,150 units) despite a drop in reticulocytosis to below 1 per cent and a rise in hemoglobin to normal or near normal values.

Normal G.O.T. values (460 and 480 units) were obtained for two patients with megaloblastic anemia prior to therapy. Ten days after the start of B_{12} therapy there was a sharp elevation in G.O.T. level to 1,080 and 1,340 units together with a marked reticulocyte response. This high level of transaminase activity was maintained

despite both the drop in reticulocytes to below 1 per cent and the increase in hemoglobin to near normal values.

A similar time relationship between reticulocyte count, hemoglobin level and transaminase level was obtained in two rabbit experiments during an episode of phenylhydrazine induced hemolytic anemia.

These preliminary results indicate that, in the limited number of patients studied, whole blood transaminase values seem to reflect a younger than normal mean red cell population due to markedly increase erythropoietic activity. Further studies are being carried out to evaluate the significance of these preliminary findings.

Exchange of Ceruloplasmin Copper with Ionic Copper[64] and Its Relation to Wilson's Disease. I. HERBERT SCHEINBERG* and ANATOL G. MORELL, New York, N. Y.

Almost all of the plasma copper of normal subjects is tightly bound to the plasma copper-protein, ceruloplasmin. This protein is deficient or absent in patients with Wilson's disease. Several investigators have postulated that the low concentration of this protein leads to the excessive absorption of dietary copper which is apparently the fundamental metabolic error underlying this illness. However, a mechanism whereby a deficiency of ceruloplasmin would result in increased absorption of copper has not yet been proposed.

In an attempt to study this problem, experiments were performed to determine whether the copper of ceruloplasmin could be reversibly dissociated from the protein. Solutions of a human plasma protein fraction of which about 85 per cent was ceruloplasmin were mixed with radioactively labelled ionic copper both in the presence and absence of ascorbic acid. Essentially all of the ionic copper was then removed by passing the solutions through columns of ion-exchange resins. Radioactivity and the concentration of ceruloplasmin copper were measured in the effluent solutions. It was found that ceruloplasmin had exchanged about half of its copper for ionic copper in the presence of ascorbic acid, although no exchange had occurred in solutions without ascorbic acid. Since this exchange was shown not to have altered the protein with respect to its blue color, copper content, or enzymatic activity, it appears that at least half of the copper-protein bonds of ceruloplasmin are reversibly dissociable.

On the basis of these results it is possible to draw tentative conclusions about the conditions required for reversible release of ceruloplasmin copper, and to consider how such a release may bear on the relation between ceruloplasmin deficiency and the disordered copper metabolism of patients with Wilson's disease.

Congenital Defects in Bilirubin Metabolism. RUDI SCHMID,* JULIUS AXELROD, LYDIA HAMMAKER, and IRA M. ROSENTHAL, Bethesda, Md., and Chicago, Ill.

It has recently been found that bilirubin is excreted in the bile as a glucuronide. *In vitro,* biosynthesis of bilirubin glucuronide was obtained by incubating crystalline bilirubin with uridine diphosphate glucuronic acid and liver microsomes. The reaction product was converted to its azoderivative, which, after paper chromatographic separation, was measured spectrophotometrically and hydrolyzed for determination of the glucuronic acid.

In homozygous animals of a mutant strain of Wistar rats, the liver microsomal enzyme system catalyzing glucuronide formation was not detectable. As a result of this biochemical lesion, the rats have a congenital nonhemolytic jaundice characterized by retention of unconjugated bilirubin in the serum but without bilirubin excretion in the urine. Secretion of bile is unimpaired, but the bile is almost colorless and contains only traces of unconjugated bilirubin. *In vitro* and *in vivo* studies demonstrated that the enzymatic defect in glucuronide formation includes, in addition to bilirubin, conjugation of o-aminophenol, anthranilic acid, and menthol, but conjugation with glycine is not impaired.

A similar defect appears to exist in children with congenital non-hemolytic jaundice. In studying serum from three such patients, all the bilirubin (20 to 35 mg. per cent) was found to be of the unconjugated type, and bilirubinuria was absent. Liver histology and conventional liver function tests, including sulfobromophthalein and cholografin excretion were normal, but the bile was almost colorless and contained only traces of unconjugated bilirubin. Glucuronide formation of menthol, salicylate, and of the metabolites of hydrocortisone was markedly depressed.

These findings indicate that impaired pigment excretion in the bile with bilirubin retention in the serum may result, both in man and in animals, from defective conjugation of bilirubin. In jaundice, due to failure in glucuronide formation, the serum contains unconjugated bilirubin and bilirubinuria is absent, whereas in obstructive jaundice, serum and urine contain conjugated bilirubin.

Elevation of Peripheral Blood Ammonia Following Muscular Exercise. ARTHUR E. SCHWARTZ, WALTER LAWRENCE, JR., and KATHLEEN E. ROBERTS,* New York, N. Y.

The hyperpnea observed during and immediately following muscular exercise has been ascribed to changes in blood pH, PCO_2, anoxia, and reflexes from exercising muscles or great veins. None of these alterations can account completely for the intensity of the hyperpnea. It has been suggested, therefore, that there is still an unrecognized respiratory stimulant contributing to the increase in ventilation during and following muscular exercise. The possible role of ammonia in this respect is worthy of consideration since it has been shown to stimulate respiration in both patients and dogs. It is known that the ammonia content of venous blood draining an exercised forearm muscle is elevated. The peripheral blood ammonia has not been measured following voluntary muscular exercise and it is not known whether the elevation would be sufficient to play any part in stimulating respiration. For these reasons studies were

carried out on human subjects, in an attempt 1) to determine if there is a significant elevation of peripheral blood ammonia following voluntary exercise or following convulsions induced by electro-shock therapy and 2) to correlate the changes in blood ammonia with changes in ventilation following voluntary muscular exercise.

The results of these studies show that peripheral blood ammonia concentration increased from the normal of 1.0 microgram per cc. to values ranging between 2.1 to 3.1 micrograms per cc. in nine patients following convulsions produced by electro-shock therapy. A similar increase in blood ammonia occurred following voluntary muscular exercise. Although hyperpnea occurred while the blood ammonia was elevated, there was not a good correlation between the actual concentration of ammonia and the degree of hyperventilation following voluntary muscular exercise.

Alkalinization by Gastrodialysis as a Means of Preventing CO_2 Narcosis. BELDING H. SCRIBNER, AUGUST G. SWANSON, and JAMES M. BURNELL, Seattle, Wash. (Introduced by Robert S. Evans).

Oxygen therapy often causes coma in anoxic patients with CO_2 retention; relief of anoxic stimulus to respiration decreases ventilation causing further retention of CO_2. If coma results from low pH rather than elevated pCO_2, then production of metabolic alkalosis prior to institution of oxygen therapy should protect the patient.

Alkalinization was attempted in a 67-year-old male with emphysema and severe cyanosis. Initial serum bicarbonate was 40 mEq. per L.; blood pH was 7.32. Cyanosis disappeared but the patient became unconscious after 15 minutes on oxygen. He regained consciousness several hours after oxygen was withdrawn. During the next 18 hours gastrodialysis against sodium carbonate using the cellophane bag technic of Schloerb was performed. Dialysis resulted in a rise in serum bicarbonate to 64 mEq. per L. and venous pH to 7.54. Sodium balance was (−) 225 mEq. due to high urinary $NaHCO_3$.

Oxygen again cleared cyanosis despite decreased ventilation. Venous pH fell to 7.40, but there was sustained improvement of sensorium. Renal acid and ammonia excretion, 20 and 24 mEq. per 24 hours, sustained the serum bicarbonate at 63 mEq. per L.

After 48 hours oxygen was discontinued. Nine hours later arterial oxygen saturation was 44.2 per cent; pH was 7.54 and bicarbonate 62 mEq. per L., as he breathed heavily at 9.6 L. per min. On 50 per cent oxygen his breathing slowed to 6.7 L. per min. Arterial oxygen rose to 83 per cent and pH fell to 7.45. On 100 per cent oxygen breathing was 3.5 L. per min., arterial oxygen 100 per cent, and pH 7.34. His sensorium remained clear.

These results suggest: 1) Loss of consciousness with oxygen therapy is related primarily to acidosis rather than elevation of CO_2 tension. 2) It may be possible to give oxygen safely to such patients if they are first made alkalotic. 3) The kidneys will sustain the resultant high serum bicarbonate when oxygen therapy slows respiration and returns blood pH to normal.

The Nature of the Metabolic Defect in Alcaptonuria. J. E. SEEGMILLER, BERT N. LADU, VINCENT G. ZANNONI, and LEONARD LASTER, Bethesda, Md. (Introduced by George K. Hirst).

Alcaptonuria is a rare, hereditary disease in which an impaired metabolism of the aromatic amino acids, phenylalanine and tyrosine, results in the excretion of large quantities of homogentisic acid in the urine. In time, the alcaptonuric patient usually develops ochronosis, a clinical condition characterized by the deposition of melanin-like pigment in the cartilages, tendons and other connective tissues. Such deposits in the joint structures may be associated with a degenerative arthritis.

It is believed that normally tyrosine and phenylalanine are catabolized in the liver through a series of enzymatically catalyzed reactions in which homogentisic acid is an intermediate compound, and that in the alcaptonuric patient there is a block in the oxidation of homogentisic acid.

An opportunity to characterize this metabolic defect more precisely was recently afforded by the necessity to repair an hiatus hernia in such a patient. A small piece of the liver was removed during surgery and an homogenate of the specimen was assayed for the various enzymes involved in tyrosine oxidation. High activities were found for the individual enzymes catalyzing the conversion of tyrosine to homogentisic acid and for those required to catabolize maleylacetoacetic acid (the immediate product of homogentisic acid oxidation) to acetoacetic acid. The enzyme required to oxidize homogentisic acid to maleylacetoacetic acid, homogentisicase, could not be demonstrated. No evidence was found for the presence of an inhibitor of homogentisicase in the patient's liver, and the addition of ferrous ion, the only known cofactor, did not result in activity. Since homogentisicase activity in human autopsy liver from a nonalcaptonuric patient was readily detected, it seems reasonable to conclude that the metabolic block in alcaptonuria is due to a lack of homogentisicase. Thus, the hereditary defect may represent a complete inability to synthesize the enzyme or an inability to produce it in adequate quantities.

Isolation Studies on Human Menopausal Gonadotrophins. ALBERT SEGALOFF* and SANFORD STEELMAN, New Orleans, La., and Houston, Tex.

Gonad stimulating hormone was concentrated from pooled postmenopausal urine by the Kaolin method of Albert. A two-fold concentration of the gonadotrophic hormone activity was achieved by extracting the solids with 10 per cent ammonium acetate and 59 per cent ethyl alcohol. This concentrate was processed by adsorption on DEAE cellulose and eluted from the columns with gradient elution by salts with a maximum concentration of 0.5 M sodium chloride and 0.1 M NaH_2PO_4. After repeated chromatography, it has been possible to isolate two materials which give preliminary evidence of homogeneity and which contain gonadotrophin hormone activity of a high order. Both of these materials contain FSH (method of Steelman and Pohley), and LH activity

(hypophysectomized rat ventral prostate). Although these preparations account for the major part of the LH activity which was recovered, it is not increased in these preparations proportionately to the increase in FSH activity. The first preparation which appears to be homogeneous by electrophoresis at two different pH's and by ultracentrifugal studies shows an approximate eight-fold increase in FSH activity over the crude Kaolin material. The second fraction, for which at present we have only paper electrophoresis evidence of homogeneity, shows an approximate fifty-fold increase in FSH activity over the crude extract.

A Model for an Immunoreaction Involving Antibody, Haptene, Platelets, and Complement, in Quinidine Purpura. N. RAPHAEL SHULMAN, Bethesda, Md. (Introduced by Thomas McP. Brown).

Patients who develop thrombocytopenic purpura due to sedormid sensitivity have in their serum an antibody which fixes complement only when the specific drug (haptene), antibody, and platelets are present together. J. S. Ackroyd determined this and other characteristics of the antibody. The antibody present in quinidine purpura reacted qualitatively in the same manner. This immunoreaction was studied in detail because it may exemplify a general type of immunologic response, with relevance to other diseases of sensitization.

Analysis of quantitative relationships between components in the antibody-quinidine-platelet complex with respect to the amount of complement fixed by the complex led to development of the following model for the molecular associations involved: First, quinidine and antibody combine. Platelets provide a surface containing sites to which antibody can attach but are not part of the antigen as previously supposed. An antibody binding one, two, or three quinidine molecules can attach to platelet sites. Complement is fixed between attached antibodies only when antibodies are in nearest-neighbor position and bind two quinidine molecules. Results calculated using equations based on this model (adaptations of Langmuir's adsorption isotherm) were consistent in all respects with experimental results.

It is of particular interest that this model presents a specific mechanism for fixation of complement by an antibody reaction.

In *in vivo* experiments on sensitized patients, thrombocytopenia occurred at concentrations of haptene too low to cause measurable complement fixation or platelet agglutination by generally employed techniques. By varying the concentration of reactants according to principles of the above model, sensitivity of these techniques can be increased; and they may be applicable in other diseases in which this type of immunoreaction is suspected.

The Enzymatic Lesions in Galactosemia. JAMES B. SIDBURY, JR., Baltimore, Md. (Introduced by Victor A. Najjar).

Galactosemia is a familial disease characterized by intolerance to dietary lactose and galactose. Galactose-1-phosphate accumulates in the tissues of these patients when there is galactose in the diet due to the demonstrated deficiency of the enzyme "P-gal transferase." The symptoms of galactosemia presumably result from this accumulation of galactose-1-phosphate, since all symptoms abate when galactose is removed from the diet.

It has been shown that galactose-1-phosphate reacts with phosphoglucomutase at approximately 1/400 the rate of glucose-1-phosphate. The mechanism of action of phosphoglucomutase has been shown to involve the transfer of phosphate from the phosphorylated enzyme to the 6 position of glucose-1-phosphate with the formation of glucose-1,6-diphosphate (the coenzyme of the reaction) and dephospho-enzyme. The glucose-1,6-diphosphate reacts with dephospho-enzyme to produce glucose-6-phosphate and regenerated phospho-enzyme. In this report galactose phosphates are shown to be inhibitory to phosphoglucomutase. This inhibition is competitive in nature and explicable within the tenets of the demonstrated mechanism of action of phosphoglucomutase. Each molecule of galactose phosphate which reacts with phosphoglucomutase consumes the transferable phosphate producing galactose-1,6-diphosphate and dephospho-enzyme. The dephospho-enzyme is rephosphorylated by any glucose-1,6-diphosphate present but this is soon depleted and dephospho-enzyme accumulates because the reaction between galactose-1,6-diphosphate and dephospho-enzyme is the excessively slow step. Thus the over-all effect is the depletion of the coenzyme glucose-1,6-diphosphate and the accumulation of the dephospho form of phosphoglucomutase which is non-reactive with glucose-1-phosphate or glucose-6-phosphate.

The phosphoglucomutase appears to be essential in a number of reactions, including metabolic steps in glycolysis, polysaccharide synthesis, nucleotide synthesis. The effect of inhibiting these reactions would be far reaching and could explain the symptomatology of galactosemia.

The Role of Glucose Oxidation in the Synthesis of Cholesterol and Fatty Acids. MARVIN D. SIPERSTEIN and VIOLET M. FAGAN, Dallas, Tex. (Introduced by Elias Strauss).

To determine how glucose oxidation influences lipid synthesis, the rate of production of fatty acids and cholesterol was measured in rat liver homogenates during stimulation of the two glycolytic pathways—Embden-Meyerhof (EM) pathway by diphosphopyridine nucleotide (DPN) and hexosemonophosphate (HMP) pathway by triphosphopyridine nucleotide (TPN). Synthesis of fatty acids and cholesterol was measured by rates of incorporation of acetate-C^{14} into these compounds.

Stimulation of EM pathway by addition of DPN slightly accelerated fatty acid synthesis and had no effect upon cholesterol production. However, when glycolysis over HMP was stimulated by addition of TPN to homogenates, an 11- to 130-fold increase in fatty acid synthesis and approximately a 100-fold increase in cholesterol synthesis was noted. Simultaneous stimulation of both pathways further increased fatty acid synthesis, but

depressed cholesterol synthesis relative to that caused by TPN alone.

Since glycolysis via either pathway yields energy, but only HMP glycolysis generates TPNH, it seemed likely that this cofactor was responsible for accelerating cholesterol and fatty acid synthesis during HMP glycolysis. This hypothesis was supported by the fact that a different TPNH generating system, isocitric dehydrogenase, was capable of substituting for HMP in enhancing lipid synthesis.

It is concluded that the hexosemonophosphate pathway may exert its major function in cellular metabolism, not simply by furnishing energy, but rather by functioning as a TPNH generating system. Since synthesis of fatty acid and cholesterol is determined in large part by availability of TPNH, the HMP pathway may provide the principal means whereby glycolysis regulates cholesterol and fatty acid metabolism.

Finally, the rate of cholesterol synthesis in the cell seems to be controlled by the relative amounts of glucose utilizing each glycolytic pathway, HMP glycolysis stimulating and EM glycolysis depressing this process.

Esophageal Catheterization Studies—The Mechanism of Swallowing in Normal Subjects and Patients with Dysphagia with Particular Reference to the Esophago-Gastric Sphincter (Vestibule). MARVIN H. SLEISENGER and JAMES H. PERT, New York, N. Y. (Introduced by Thomas P. Almy).

By means of polyethylene catheters pressures have been recorded simultaneously from three sites in the esophagus by means of electromanometers and a Sanborn recorder. A pump maintained a flow of 3 cc. water per hour through the catheters. Catheter positions were checked by fluoroscopy and documented with spot films. Attention was focused upon motility during deglutition. Twenty-two studies were performed on five normal subjects, six patients with cardiospasm and six individuals with intermittent "functional" dysphagia.

There are distinctive swallowing patterns for the upper 8/9 of the esophagus (body) and lower 1/9 (vestibule). Five components of the body wave are identified; the largest represents peristalsis which usually attains pressures of 15 to 20 cm. water and lasts 8 to 10 seconds. This wave travels at a rate of 2.0 to 3.0 cm. per second and stops at the vestibule.

The deglutition complex of the vestibule, the terminal 2 to 5 cm. of esophagus, is characterized by brief relaxation followed by contraction with a rise in pressure to 15 to 20 cm. water which lasts for 20 seconds. Strikingly, the pressure will suddenly drop, in a second or less, to baseline. This pressure rise begins as peristalsis is progressing down the body. The failure of peristalsis to enter the vestibule, the height of pressure reached and its sudden relaxation during deglutition are consistent with the action of a sphincter.

In cardiospasm the narrowed distal segment corresponds to the vestibular sphincter. It has a high resting pressure, fails to relax in a coordinated manner during swallowing and generates inordinately high pressures, occasionally reaching 100 cm. water for as long as 15 minutes. Other abnormalities of this condition include: failure of progression of peristalsis in the body and the responses of both body and vestibule to autonomic drugs.

The esophagus in patients with "functional" dysphagia demonstrates progress of peristalsis in the body and also reacts normally to drugs but has statistically higher vestibular pressures for longer periods of time than is normal.

The Estimation of Non-thyroxine Iodine Released by the Thyroid Gland in Hyperthyroidism. D. WARD SLINGERLAND and BELTON A. BURROWS,* Boston, Mass.

Although small amounts of organic iodide compounds other than thyroxine have been identified in sera of hyperthyroid patients, the major component of circulating organic iodine is thyroxine. The present study indicates that the hyperthyroid gland may release non-thyroxine iodine, either as iodide or some rapidly metabolized organic iodine compound, in amounts approaching the secretion of thyroxine iodine.

Following a six to eighteen-day period of equilibration with a tracer dose of radioiodine, the effect of antithyroid compounds and iodides on thyroidal radioiodine, serum protein-bound radioiodine and chemical iodine, and urinary radioiodine excretion was determined. With Tapazole®, urinary radioiodine excretion was increased in normal subjects several times more than would result from inhibition of thyroid uptake of radioiodide recirculated from body tissues, but in hyperthyroid subjects the increase in urinary radioiodine could come entirely from peripheral hormonal degradation. As one possible explanation for this finding, in normal subjects there may be interference with reutilization of radioiodine released by intrathyroidal deiodinating mechanisms, but in hyperthyroidism intrathyroidal recycling of iodine may not occur.

As antithyroid compounds were continued, a decline in urinary radioiodine excretion relative to serum protein-bound radioiodine levels was noted. Occasionally, administration of iodides in addition to Tapazole® resulted in immediate reduction in urinary radioiodine excretion amounting to more than one-half previous values. Concomitant slowing in the slope of thyroidal radioiodine, with a constant slope in serum protein-bound radioiodine and chemical iodine values, suggested an inhibition of thyroid release of radioiodine in some form other than thyroxine.

Secretion of significant amounts of non-thyroxine iodine, resulting from incomplete intrathyroidal deiodination of organic iodine compounds, incomplete reutilization of iodide released by such deiodinating mechanisms, or incomplete iodination of organic iodine compounds, may be of primary importance in the pathogenesis of hyperthyroidism and the response to antithyroid compounds and iodide.

A Mechanism for Hypoalbuminemia in Patients with Ulcerative Colitis and Regional Enteritis. JESSE L. STEINFELD, JOHN D. DAVIDSON, and ROBERT S. GORDON, JR., Bethesda, Md. (Introduced by A. I. Mendeloff).

The hypoalbuminemia frequently found in patients with ulcerative colitis and regional enteritis has been presumed to be due largely to poor absorption of essential nutrients. It has been suggested that loss of protein into the lumen of the bowel might also contribute to the hypoalbuminemia in these patients.

By immunochemical methods both albumin and gamma globulin have been demonstrated in the succus entericus of a patient with regional enteritis whereas neither was detectable in specimens from normal individuals. Electrophoretic analyses revealed both hypoalbuminemia and hypogammaglobulinemia in two patients with regional enteritis.

A single injection of 50 mgm. of human serum albumin labeled with 100 microcuries of I^{131} has been administered intravenously to patients with regional enteritis or ulcerative colitis and to patients without gastrointestinal disease; blood samples, stools and urine were collected for periods up to four weeks. Whereas in patients without gastrointestinal disease, 0.1 to 1.0 per cent of the total daily I^{131} excretion was found in the feces, patients with diarrhea due to regional enteritis or ulcerative colitis excreted 10 to 50 per cent of the radioactivity in the stools. Data on the stool:urine partition of radioactivity following the intravenous injection of iodide^{131} and I^{131}-labeled albumin in individuals without gastrointestinal disease before and after the induction of diarrhea will be presented.

Administration of tracer quantities of I^{131}-labeled human serum albumin to patients with regional enteritis and ulcerative colitis has revealed their total exchangeable albumin to be sub-normal. However, albumin turnover (grams per kilogram of body weight per day) was greater than normal; the fraction of the total albumin pool renewed each day was approximately twice normal. Since albumin turnover is actually increased in these patients it is extremely unlikely that poor absorption of protein precursors is the principal cause of the hypoalbuminemia.

The Rate of Degradation of Thyroxine in Patients with Thyroid Disease after Therapy. KENNETH STERLING,* Syracuse, N. Y.

The disappearance curve of plasma radioactivity after intravenous injection of I^{131} labeled l-thyroxine was employed to determine the rate of hormone degradation or removal. Studies were carried out before and after therapy in patients with thyroid disease.

Thyroxine degradation was slower than normal in untreated myxedema, and faster than normal in thyrotoxicosis. Follow-up studies were carried out after appropriate therapy and attainment of the euthyroid state. Euthyroidism was evaluated by clinical appearance, basal metabolic rate, and plasma protein bound iodine concentration. The initially abnormal disappearance curves had usually reverted to normal or nearly so when patients were restudied two months to several years after treatment.

In five myxedematous patients on prolonged replacement therapy, the degradation rate had returned to the normal range or slightly below. In contrast, no alteration in the disappearance curve occurred in subjects followed for one to three weeks after rapid treatment with triiodothyronine.

After treatment of thyrotoxicosis with radioiodine, surgery, or antithyroid drugs, the degradation rate became normal or approximately normal in seventeen subjects. In one instance the rate remained markedly accelerated despite clinical euthyroidism. In two cases of post-radioiodine hypothyroidism, the rate was as slow as that seen in spontaneously occurring myxedema.

The results suggest that the rate of removal of circulating thyroxine is a significant parameter of thyroid hormone metabolism.

Observations on Deoxyribonucleic Acid Synthesis by Human Bone Marrow Cells in Vitro. E. DONNALL THOMAS, Cooperstown, N. Y. (Introduced by James Bordley, III).

By available criteria of cellular viability, bone marrow *in vitro* appears to survive at best for only a few days. The evaluation of minor changes in cell count during this short period is difficult and represents a balance between cell production and cell destruction. Deoxyribonucleic acid (DNA) has been shown to be metabolically stable, and measurement of the rate of its formation is considered a direct function of cell proliferation. Isotopic formate is incorporated into the methyl group of thymine by bone marrow cells. Since thymine occurs only in DNA, it appears that DNA synthesis can be followed by the direct isolation of thymine without the necessity of separating DNA from ribonucleic acid. This paper describes studies of DNA synthesis by normal human bone marrow *in vitro* by measuring the rate of incorporation of C^{14}-formate into thymine.

It was found that DNA synthesis continues at an approximately constant rate for several hours. The optimal formate concentration was found to be 0.5 micromole per ml. and optimal pH approximately 8. The presence of serum is essential for optimal DNA synthesis, and it cannot be replaced by currently available synthetic media. The effect of X-irradiation on normal human bone marrow was studied. Results indicate that as little as 200 r of X-ray interferes with DNA synthesis. However, heme synthesis was unaffected by doses less than 1,000 r. Data concerning the time factor in the effect of X-ray on human bone marrow will be illustrated.

The method appears to be reproducible and to make it possible to follow rates of DNA synthesis over very short periods of time and with small amounts of marrow. It should prove useful in studying many of the problems concerned with the proliferation and viability of bone marrow cells.

Studies on a Physiologically Important Enzymatic Reaction: Adrenal 11-β-Hydroxylation. GORDON M. TOMKINS, Bethesda, Md. (Introduced by Joseph E. Smadel).

The introduction of an 11-hydroxyl group into the steroid nucleus is an essential step in the biosynthesis of the physiologically active adrenal steroids cortisol (compound F), corticosterone (compound B), and aldosterone. Information about this reaction is, therefore, of the utmost importance.

By the use of partially purified cell-free extracts of the adrenal and other organs, we have demonstrated that at least two distinct enzymes are involved in this process. One of these is present only in adrenal mitochondria. The other, however, is much more widely distributed and has been detected not only in the adrenal, but also in the lung, spleen, liver, and serum.

We have observed that amphenone B, used clinically by Hertz and his associates to depress adrenal cortical function, is a potent inhibitor of the 11-hydroxylating enzyme system. Diphenyl propyl acetic acid (S.K.F. no. 2314), an inhibitor of drug metabolism, and chemically somewhat similar to amphenone, has also been found to block adrenal 11-hydroxylation *in vitro*. Furthermore, it has been found that the activity of the hydroxylating enzyme system is inversely proportional to the ionic concentration of the medium even within physiological limits. Although it is not possible at this time to assess whether or not this inhibition by salts is of any homeostatic significance, it is interesting to note that the direction of the effect (that is, inhibition of hydroxylation by high ionic strength) would serve to maintain a constant intracellular ionic environment by inhibiting the synthesis of salt-retaining hormones.

It would appear that a detailed examination of the enzymatic hydroxylating system might provide insight into chemical mechanisms which are of physiological and clinical importance.

Perfusion Studies of the Human Placenta: Hormonal Effects on Citric Acid Metabolism. PHILIP TROEN and EDWIN E. GORDON, Boston, Mass. (Introduced by Samuel L. Gargill).

The factors controlling placental metabolism and the role of human chorionic gonadotropin (HCG) in this process have not been established. In initial studies of this problem the metabolic activity of the placenta has been determined utilizing perfusion techniques. Villee and associates have shown that the utilization of citric acid by placental homogenates is enhanced by estradiol stimulation of a DPN-dependent isocitric dehydrogenase. Accordingly, freshly obtained human placentas were perfused for several hours with a modified Tyrode's solution containing citric acid. Hourly measurements were made of citric acid utilization and α-keto acid production. The effect of hormones added to the perfusing fluid was studied in four groups of three placentas each: 1) control, 2) estradiol-17β, 3) HCG, 4) estradiol and HCG combined.

The control group had an average total utilization of 854 mgm. citrate per placenta and an average total accumulation of 23.4 mgm. of α-keto acid (expressed as α-keto-glutaric acid) per gland. Addition of either estradiol or HCG separately to the perfusate did not result in a significant difference from these total values. However, when estradiol and HCG were added simultaneously there was a significant increase in average total citrate utilization to 1615 mgm. per gland. The hour-to-hour values also revealed significant increasing utilization of citrate by the placentas in this group. In addition, the total accumulation of α-keto acid dropped to an average total of 13 mgm. per gland in this group.

These data demonstrate metabolic activity persisting in human placentas for at least eight hours of perfusion. An effect of HCG on such metabolic activity has been shown for the first time. This methodologic approach permits study of the factors controlling placental metabolism and may provide clarification of the endocrine functions of the human placenta.

The Fatty Acids of Normal Plasma and of Arteriosclerotic Plaques. NAIP TUNA, LOIS RECKERS, and IVAN D. FRANTZ, JR.,* Minneapolis, Minn.

The literature contains suggestions that the saturated fatty acids, by virtue of their relative insolubility, may deposit preferentially in the arterial wall, thus playing a special role in the development of atherosclerosis. In an attempt to settle this question, we have analyzed the fatty acids of total lipides and of cholesterol esters from pooled plasma of normal persons, and from atheromatous plaques obtained from autopsy material. The total lipides were extracted with chloroform-methanol. The cholesterol esters were separated from other lipides by silicic acid chromatography. The fatty acids were first partially separated by counter-current distribution. The peaks were then analyzed by iodine value, alkali isomerization, paper chromatography before and after hydrogenation, and vapor phase chromatography.

The most important conclusions to be drawn from these analyses are as follows: (1) The fatty acid spectrum of the total lipides of plaques is very similar to that of serum. Both contain about 30 per cent of oleic acid, with slightly smaller amounts (20 to 25 per cent) of linoleic and palmitic acids. In addition to these constituents, the mixture contains 4 to 7 per cent each of stearic, arachidonic, and C_{18} unsaturated acids, as well as traces of other components. (2) The fatty acids of cholesterol esters include about 40 per cent of linoleic acid. Otherwise they do not differ greatly from the fatty acids of total lipides. (3) The cholesterol esters of serum and plaques are quite similar.

Although it is still possible that "essential" fatty acids are important in the prevention of atherosclerosis, the present findings indicate that any such effect is not due to their inability to participate in plaque formation.

Zinc Metabolism in Post-Alcoholic Cirrhosis. BERT L. VALLEE,* WARREN E. C. WACKER, and JEREMIAS H. R. KAEGI, Boston, Mass.

Iron in hemochromatosis and copper in Wilson's disease are implicated in the pathogenesis of these diseases, both of which ultimately lead to cirrhosis. Zinc, another transition-element, participates in the dehydrogenation of ethanol. This led to studies of zinc in post-alcoholic cirrhosis. In normal individuals ~500 μg. per 24 hours of directly reacting zinc is excreted. Elimination of zinc in the urine apparently varies with the functional capacity of the post-alcoholic liver. "Zincuria" from 750 to 2,500 μg. per 24 hours has been observed in individuals with cirrhosis and hepatomegaly; apparently the first authenticated instance of primary "zincuria" without albuminuria. One terminal, jaundiced individual, however, with a small liver, ascites, serum-zinc 49 μg. per cent, and BSP 61 per cent, excreted ~50 μg. of zinc in 24 hours. Oral zinc induced transient changes in zinc excretion, and after ten days the BSP was 53 per cent. Another patient with marked hepatomegaly presented a different pattern: Serum-zinc was 77 μg. per cent, urinary zinc excretion was about 800 μg. per 24 hours and the BSP was 32 per cent during the control period. Oral zinc abruptly *decreased* urinary zinc concentration to ~400 μg. per 24 hours. This level was maintained below the mean of the normal series throughout the experimental period. Serum-zinc rose to within normal limits. BSP was 10 per cent after ten days, remaining at this level.

The participation of zinc in the dehydrogenation of ethanol, in the metabolism of post-alcoholic cirrhosis and the tendency of physiological quantities of zinc to restore normal biochemical patterns in the individuals studied emphasizes the unsuspected participation of this transition-element in another pathologic process, characterized by severe cicatrization in its terminal stages.

Flame Spectrometry, Magnesium and the Uremic State. WARREN E. C. WACKER, Boston, Mass. (Introduced by Gustave Dammin).

Somnolence and coma are frequent manifestations of the uremic state. The biochemical mechanisms leading to these clinical changes are obscure. The known pharmacological effect of magnesium bears a resemblance to certain features of this syndrome which is characterized by electrolyte disturbances.

The development of flame photometry has facilitated the study of sodium and potassium, but the chemical methods for magnesium have remained cumbersome. The physiology of magnesium and its clinical implications is not understood as well as that of the alkali metals, but the function of magnesium in enzymology has been studied intensively.

The validation of a flame-spectrometric method for magnesium has permitted the examination of this element in renal failure. The procedure has a repeatability of 1.9 per cent with excellent recovery. The normal serum magnesium follows a Gaussian distribution with a mean of 2.05 ± 0.18 mEq. per L.

In several patients serum magnesium concentrations varying from 3.5 to 5.5 mEq. per L. have been observed in the presence of clinical and chemical changes characteristic of renal insufficiency. While anuria was a common feature, hypermagnesemia also occurred in the presence of a normal volume of urine. Hemodialysis when performed without magnesium in the dialyzing fluid significantly lowered the serum magnesium concentration, in accord with evidence that a fraction of this element is protein-bound. Electrocardiographic changes reported to be typical of hypermagnesemia were detected and found reversible with dialysis.

These studies indicate distinct abnormalities in magnesium metabolism of patients with some types of renal failure. The recognition of this change has important implications in the diagnosis and management of renal failure, particularly of the acute, reversible type where it may constitute a criterion for hemodialysis.

Urea Breakdown in Normal Man. MACKENZIE WALSER and LEONARD J. BODENLOS, Bethesda, Md. (Introduced by Gilbert H. Mudge).

In normal subjects, the rate of urea breakdown, heretofore assumed to be negligibly small, has been determined by injecting intravenously trace quantities of urea labeled with either C^{14} or N^{15}. Between the second and eighth hours after injection, blood urea isotope concentration declined exponentially in five experiments with N^{15}-urea and in six out of seven experiments with C^{14}-urea, indicating that appreciable reincorporation of labeled atoms into newly formed urea during this period occurred in only one instance. Therefore, it was possible to calculate the rate of urea production as the product of the pool size and the turnover rate, after making allowance for excess isotope excreted during the first two hours. Urea production exceeded the simultaneous rate of urea excretion by 15 per cent to 55 per cent (mean 23 per cent). In one week, 79.5 per cent of a dose of 25 mg. excess N^{15} (given as urea) was excreted in the urine as urea, 0.4 per cent as ammonia, and 9.7 per cent as undetermined nitrogen. Urinary creatinine and uric acid were not enriched. Cumulative urinary recovery of C^{14}-urea averaged 70 per cent within 46 hours (four subjects) and 81 per cent within 72 hours (three subjects). Reincorporation of label into urea occurred with C^{14} as well as with N^{15} as indicated by the fact that 1 per cent of the dose of C^{14} was recovered as urea between 72 and 96 hours after injection.

Oral chlortetracycline, oxytetracycline, and succinylsulfathiazole usually reduced but did not eliminate breakdown. Oral Neomycin preparation for 48 hours resulted in urinary recovery of 96 ± 2 per cent (s.e.) of C^{14}-urea as urea in six subjects in 72 hours.

Thus urea breakdown by gastrointestinal bacteria constitutes a significant pathway of nitrogen catabolism, resulting in the potential release of about 2 grams a day of ammonia into the portal circulation.

Identification of a New Group A Streptococcal Desoxyribonuclease: The Antibody Response in Scarlet Fever and Acute Rheumatic Fever. LEWIS W. WANNAMAKER, New York, N. Y. (Introduced by Maclyn McCarty).

Strain variation among Group A streptococci has been explored by comparing electrophoretic patterns of concentrates of culture supernates. These studies revealed the presence of two distinct streptococcal enzymes, both of which depolymerize desoxyribonucleic acid. The two desoxyribonucleases migrate differently on starch block electrophoresis and are distinguishable serologically by differential antibody neutralization. They are designated here as DNASE I and DNASE II.

DNASE I resembles that previously described by McCarty and can be identified in preparations from the strain used in his studies. DNASE II appears to be more generally distributed among strains of Group A streptococci. Moreover, antibody studies suggest that DNASE II is a more frequently encountered streptococcal antigen than DNASE I. None of the sera from 12 normal adults had detectable antibody to DNASE I, whereas all showed some antibody to DNASE II, usually in titers between 1:20 and 1:80. Thus, antibody to DNASE II resembles antistreptolysin O in its almost universal distribution in low titers among normal adults.

Acute and convalescent sera from 21 patients with scarlet fever were examined. Fourteen showed a rise in antibody titer to one or both of the enzymes. Six showed a rise to DNASE I, whereas 13 showed a rise to DNASE II.

In patients with acute rheumatic fever, antibody to DNASE II was demonstrated more frequently and at higher levels than antibody to DNASE I. No antibody to DNASE I was detected in 12 of 38 such patients, and only 9 showed titers of 1:80 or greater. In contrast, 34 of the 38 patients showed titers of 1:320 or greater to DNASE II. These data suggest that, like antistreptolysin O, antibody to this new desoxyribonuclease (DNASE II) may be a useful index of recent streptococcal infection among patients in whom the diagnosis of acute rheumatic fever is being considered.

Depression of the Total Body Respiratory Quotient in Human Malignant Disease. DONALD M. WATKIN, Bethesda, Md. (Introduced by Charles G. Zubrod).

Total respiratory quotients (R.Q.'s) were measured to estimate the proportion of fat in the nutrient mixture metabolized by subjects with and without malignant disease. High fat values in subjects with progressive neoplasia had previously been computed from balance study data using Lavietes' formula. R.Q. measurements were made 15 hours post prandial at intervals of several days in each subject. Room air was inspired. Expired air was collected for 36-minute periods in Douglas bags or metered and aliquoted in a Franz-Müller gasometer. Samples were analyzed for oxygen and carbon dioxide in Beckman and Cambridge instruments, respectively. Under these conditions the R.Q.'s of healthy adults averaged 0.786; of subjects chronically ill with non-malignant disease, 0.784; of subjects with clinically inactive malignancies, following surgery, radiation or endocrine manipulations, 0.801; but of adequately nourished subjects with progressive malignant disease, 0.693. In subjects initially demonstrating low R.Q.'s and rapidly progressing neoplasms, hypophysectomy with replacement corticoid therapy, corticoid therapy alone and large doses of radiation to extensive body areas produced temporary elevations in R.Q. and temporary objective cessation of tumor growth. Upon relapse the R.Q.'s returned to control values. The data suggest that subjects with advanced neoplasms metabolize more fat than normal adults, subjects with non-malignant chronic diseases and subjects with arrested malignancies. They are compatible with interpretations of balance data previously derived using Lavietes' formula. Reduction of the total body basal R.Q. implies the existence of a metabolic process associated with but not necessarily dependent upon the anatomical neoplasm. This process may be modified by hypophysectomy, corticosteroids and extensive body radiation.

Effect of a Carbonic Anhydrase Inhibitor, Acetazoleamide (Diamox®), on Cerebral "Ammonium" Metabolism of Patients With and Without Hepatic Cirrhosis. LESLIE T. WEBSTER, JR., and JEROME KLEINERMAN, Cleveland, O. (Introduced by George J. Gabuzda).

Diamox® may induce impending hepatic coma (mental confusion and a flapping tremor) associated with increased antecubital venous blood "ammonium" concentrations in susceptible patients with hepatic cirrhosis. Accordingly, ammonium metabolism of the brain was evaluated twice before and twice within 60 minutes after injecting 500 mg. of Diamox® intravenously into 8 patients with and 8 without hepatic cirrhosis. Arterial and superior jugular bulb venous blood samples were analyzed for "ammonium" (Conway), glucose (Somogyi) and oxygen (Van Slyke). Cerebral blood flows were determined before and after Diamox® in 6 patients of each group by the nitrous oxide technique (Kety).

In patients without liver disease, changes in arterial "ammonium," glucose, and oxygen were not significant after Diamox®. Cerebral blood flow increased significantly ($p = .05$) but cerebral uptakes of "ammonium," glucose, and oxygen were not consistently affected.

In contrast, patients with cirrhosis exhibited significantly increased arterial and jugular blood "ammonium" concentrations after Diamox® (24 per cent above the control, $p < .01$). A significant cerebral uptake (mean = 6.2 micrograms per 100 gm. tissue per minute; $p < 0.05$) of "ammonium" occurred also although there was neither a significant uptake nor release during the control period. Neither arterial glucose concentration, oxygen content, nor cerebral uptakes of glucose or oxygen were changed, but the cerebral flow again increased ($p = .05$) after Diamox®.

Increased blood "ammonium" concentrations noted after Diamox® administration to patients with cirrhosis are

probably of endogenous origin because practically none of the nitrogen contained in this drug is released. The cerebral "ammonium" uptake indicates that "ammonium" liberation from the brain does not account for the increased arterial blood "ammonium" concentrations observed. A cerebral "ammonium" uptake is associated with impending hepatic coma induced by a nitrogen load. Whether or not the cerebral "ammonium" uptake following Diamox® administration is related to the neurological deterioration induced by this drug requires further investigation.

Inhibition of the Bactericidal Activity of the Properdin System with Specific Bacterial Antibodies. RALPH J. WEDGWOOD, Cleveland, O. (Introduced by William M. Wallace).

The nonspecific bactericidal activity of normal human serum against Gram-negative bacteria requires properdin and has been shown to be distinct from immune mechanisms requiring antibody. This communication shows that the bactericidal activity of properdin can be specifically inhibited by antibodies to the bacterium.

Serum, in which bactericidal activity has been markedly diminished by almost complete properdin depletion, may contain antibodies to the somatic antigen of *Sh. dysenteriae.* Removal of such antibodies by adsorption with the antigen restores bactericidal activity against *Sh. dysenteriae,* but does not affect bactericidal properties against a heterologous organism—*E. coli.* Similarly, the addition of specific somatic antibodies to intact serum decreases bactericidal activity to the homologous but not to the heterologous organism. Within limits, a direct stoichiometric relationship exists between the amount of antibody, which inhibits, and the amount of properdin, which is needed, to produce bacteriolysis. This relationship is distinct from pro-zone phenomena.

It is generally considered that specific antibodies to Gram-negative bacteria may be bactericidal. The findings presented here indicate that specific antibody to the somatic antigen of *Sh. dysenteriae,* rather than being bactericidal, may actually inhibit the normal nonspecific bactericidal activity of human serum. This inhibition of the bactericidal activity of the properdin system by antibody seems to be immunologically specific and probably represents competition between properdin and antibody for similar sites on the bacterium. These studies offer a new approach for determining this site of interaction of the properdin system with the bacterium itself.

The Effect of Total Adrenalectomy in a Patient with Chronic Massive Ascites Caused by Post-Necrotic Cirrhosis. EMILE E. WERK, JR., JEROME GIUSEFFI, JR., PAUL U. LARSON, DAVID W. ELLIOTT, and YOICHI OIKAWA, Cincinnati, O. (Introduced by Leon Schiff).

Bilateral adrenalectomy was performed June, 1956, in a 28-year-old man to alleviate "maximum" ascites which had existed 14 months as the predominant manifestation of post-necrotic cirrhosis.

Two weeks post-operatively while receiving liberal dietary sodium and cortisone acetate (E) 37.5 mg. daily, the patient gained weight rapidly. Urinary output averaged 700 ml. per day and urinary sodium was 8 mEq. per day. Daily E was reduced to 25 and 12.5 mg. and sodium intake decreased. Weight remained constant. Urinary sodium ranged from 20 to 100 mEq. per day. On 8 mg. E per day weight diminished, urinary volume and sodium increased but anorexia and depression developed.

In August, a cellulitis and marked adrenal insufficiency intervened and produced a profuse diuresis which high cortisone dosage plus antibiotic administration reversed. A sustained sodium and water diuresis then ensued over the next three months. Weight decreased 27 Kg. and no ascites or edema was perceptible. Appetite and strength improved. E dosage varied from 18.75 to 50 mg. daily. At intervals prednisone was added. During the greater part of this period estimated sodium intake was approximately 100 mEq. per day, fluid intake 1,700 ml. per day and urinary volume and sodium averaged 1,200 ml. and 120 mEq. per day, respectively. Ascites reaccumulated for a brief period in December when patient was allowed a free sodium and water intake and received hydrocortisone 25 mg. daily.

Urinary aldosterone levels (performed by Dr. Edgar Gordon) were 70 mcg. per day pre-operatively and zero one month post-operatively.

Thus adrenalectomy permitted loss of ascites and improvement with the patient maintained on moderate sodium intake and replacement cortisone. However, a defect in water and salt retention remained which was prominent two months post-operatively and lessened following an acute infection but persisted after ascites had completely disappeared.

A Distinction Between the Anticoagulant and Antithrombotic Effects of Dicumarol®. STANFORD WESSLER,* JONATHAN D. BALLON, and JAY H. KATZ, Boston, Mass.

The cardinal problem in Dicumarol® therapy is the determination of the amount of drug necessary to inhibit thrombosis without precipitating hemorrhage. The therapeutic dose, however, is gauged by laboratory tests which reflect specifically a coagulation defect: a parallel antithrombotic effect has been assumed. In dogs the systemic infusion of serum produces a temporary hypercoagulable state during which massive thrombosis is routinely induced in areas of retarded blood flow. This technic permits independent measurement of coagulation defects and thrombus formation. In short term experiments, serum fractions were obtained from 13 donor dogs in which the one-stage overall plasma prothrombic activity had been reduced by Dicumarol® to values ranging from 25 to 6 per cent of the control values. These serum fractions were then infused into 13 additional animals in which the prothrombic activity had been comparably depressed by Dicumarol®. The coagulation defect in both the donor and recipient animals was significant; yet, thrombosis was observed in each recipient animal. Moreover, the

partial inhibition of thrombosis found in 3 of the 13 animals could not be correlated with the one-stage overall prothrombic activity, prothrombin or convertin activities of either donor or recipient dogs. A dichotomy was thus demonstrated between the coagulation defect and the antithrombotic effect produced by Dicumarol®. Moreover, the one-stage overall prothrombic activity, prothrombin and convertin activities did not accurately reflect this antithrombotic activity of the drug. These results provide an experimental counterpart to clinical experience in which thrombosis has occurred in Dicumarol®-treated patients exhibiting a marked interference with the coagulation mechanism. These findings also reinforce the need for a more appropriate measure of the antithrombotic activity of Dicumarol® than is currently available.

Water Diuresis and Antidiuretic Mechanisms in Diabetes Insipidus and Hepatic Cirrhosis. ABRAHAM G. WHITE, New York, N. Y. (Introduced by Milton Mendlowitz).

The occurrence in diabetes insipidus of: (1) subnormal diuresis after hydration, (2) edema, and (3) ascites (produced experimentally), points to an important physiological role of an extra-neurohypophysial antidiuretic mechanism in this syndrome. That an extra-neurohypophysial antidiuretic mechanism may play a major role in the water retention of hepatic cirrhosis has been suggested by studies of water diuresis, Pitressin® antidiuresis, alcohol diuresis and serum antidiuretic activity in this disease.

To determine whether or not the aforementioned extra-neurohypophysial antidiuretic mechanisms are similar in these two disease states, studies were made of water diuresis following intravenous hydration with 5 per cent glucose in water (10 ml. per min.) in three patients with diabetes insipidus and four with hepatic cirrhosis. In three additional patients with diabetes insipidus data were obtained on water diuresis following oral hydration with 1,500 ml. water. Fourteen normal subjects served as controls, seven for intravenous hydration, and seven, oral hydration.

Following intravenous hydration, the four patients with cirrhosis reached a peak diuresis in 94.5 ± 27.5 min. with a mean peak flow of 11.9 ml. per min.; the three patients with diabetes insipidus did not attain a sharp peak of diuresis, and had a mean maximal urinary flow of 7.6 ml. per min. Control subjects attained a peak in 85.1 ± 32.3 min., with a mean peak flow of 12.0 ml. per min.

After the ingestion of 1,500 ml. of water within 25 min., control subjects reached a mean peak diuresis of 15.2 ml. per min., while patients with diabetes insipidus attained a mean maximal flow of 6.3 ml. per min.

The subnormal diuretic responses of patients with diabetes insipidus following both oral and intravenous hydration are interpreted as confirming the presence of extra-neurohypophysial antidiuretic mechanisms. The differences in the diuretic responses between patients with diabetes insipidus and those with cirrhosis suggest differences in the antidiuretic mechanisms operative in these two disease states.

Radioactivity of Human and Cattle Thyroids Associated with Fallout. MARGARET R. WHITE and HARDIN B. JONES, Berkeley, Calif. (Introduced by John H. Lawrence).

The level of radioactive iodine in human thyroids obtained at autopsy has been followed since January, 1956, and that in thyroids of cattle slaughtered in the San Francisco Bay area since February, 1955.

No individual human thyroid of the 155 studied during this period contained enough I^{131} to be detected even by sensitive counting techniques. It was possible, however, to obtain an outside limit of the I^{131} concentration in human thyroids and to estimate the maximum possible level. All the human thyroids corresponding to the period of constant and high I^{131} level in range cattle thyroids (June to October, 1956) were recounted after decaying through a minimum of four I^{131} half-lives. It was estimated that the average activity attributable to I^{131} in the human thyroids was 0.0006 mμc. per gm. (1/1,000th of the average level observed in range cattle thyroids), that there was only one chance in 1,000 that human thyroids could have received a dose exceeding 0.001 rep, and that the probable value was 0.00016 rep.

Three periods of sharply elevated radioactivity were noted in range-fed cattle thyroids. (1) The Nevada series in the spring of 1955 produced a maximum radioactivity to an individual thyroid of 6.4 mμc. of I^{131} per gram of wet tissue and delivered a total dose to range-fed cattle thyroids for the period of elevated activity of 1.0 rep. (2) The Russian test in March of 1956 produced a maximum radioactivity of 1 mμc. per gm. and a total dose of 0.2 rep. (3) The Bikini tests in the spring and summer of 1956 produced a maximum radioactivity of 2.67 mμc. per gm. and a total dose of 1.3 rep. The thyroids of feed-lot-fed cattle showed much lower values (generally being near background), implicating the range-plant food as the only probable source of radioactivity.

Effect of Thiamin Deficiency on Human Erythrocyte Metabolism. STANLEY J. WOLFE and MYRON BRIN, Boston, Mass. (Introduced by Charles S. Davidson).

Augmented oxygen consumption of mammalian erythrocytes by methylene blue has been demonstrated to result from hexose monophosphate shunt activation. As this pathway cycles and after the first carbon of the hexose molecule is oxidized to CO_2, the second and then the third carbons successively assume the carbon-1 position and are subsequently recoverable as CO_2. A fixed ratio exists normally between carbon-1 and carbon-2 recovery. By labelling carbons 1 and 2 of glucose with C^{14}, blocking of the cycle was demonstrated in thiamin-deficient rat erythrocytes, as evidenced by greatly reduced carbon-2 recovery and increased pentose accumulation.

Erythrocytes from patients with thiamin deficiency, Wernicke's encephalopathy with ophthalmoplegia, showed similar alterations.

Erythrocytes were incubated (Warburg) 3 hours at 38° C under air with methylene blue and glucose labelled (C^{14}) on carbon 1 or 2 (separate flasks). Recoveries of $C^{14}O_2$ determined separately for the 2 substrates were expressed as percentages of C^{14} counts added. Oxygen consumption and pentose accumulation were determined.

Erythrocytes from 25 controls (normals and hospital convalescents) showed mean carbon-1 recoveries of 35.6 ± 4.1%, and carbon-2 of 16.7 ± 2.2%. Mean oxygen consumption was 108.0 ± 11 μl. and pentose accumulation 131 ± 13 μg. per flask. Erythrocytes of 8 Wernicke patients pre-treatment showed mean carbon-1 recovery of 36.7 ± 3.6% and carbon-2 of 10.2 ± 2.1%. Mean oxygen consumption was reduced slightly (98.4 μl.) and pentose accumulation increased (169 μg. per flask). *In vitro* addition of thiamin or cocarboxylase produced increased carbon-2 recoveries. After thiamin treatment, with remission of ophthalmoplegia, the patients' erythrocytes showed 31.7 ± 4.3% carbon-1 recovery, 13.8 ± 2.1% carbon-2, 105.5 μl. oxygen consumption and 148 μg. pentose accumulation.

Significantly reduced carbon-2 recovery ($p < 0.01$) without change in carbon-1 recovery associated with increased pentose accumulation indicates interference with glucose oxidation at the transketolase step after removal of the first carbon as CO_2. This reduced hexose recycling results from insufficient thiamin as cotransketolase.

Reduction of Reactive Hyperemia Blood Flow of the Foot by Tobacco Smoking. J. Edwin Wood and Jay D. Coffman, Boston, Mass. (Introduced by Robert W. Wilkins).

In patients with and without vascular disease foot blood flows were measured with a constant temperature (89° F) water plethysmograph at 15-second intervals for two minutes following five minutes of arterial occlusion of the foot. The subject then smoked two cigarettes at his usual rate. Five minutes after smoking began, arterial occlusion was reapplied and the measurements were repeated. By suitable calculation the total volume of blood that flowed through the foot during the two minutes of reactive hyperemia while smoking was compared to that without smoking.

Of 19 subjects studied 20 times in a warm (83° F) environment, 13 showed a definite decrease in reactive hyperemia blood flow (RHBF) during smoking. Control (warm) RHBF averaged 15.3, ranging from 6.3 to 41.2 cc. per 100 cc. of foot. During smoking RHBF averaged 10.1, ranging from 3.0 to 36.2 cc. per 100 cc. Seven subjects showed no change in RHBF while smoking.

Eight subjects were restudied in a cool (68° F) environment which produced a definite decrease of "Control" RHBF. Cool control RHBF averaged 10.2, ranging from 5.5 to 16.6 cc. per 100 cc., and during smoking RHBF decreased in six of the eight and averaged 5.0, ranging from 1.3 to 10.5 cc. per 100 cc.

RHBF was measured simultaneously in both feet of three patients with unilateral sympathectomy in the warm and cool environment. In five of six studies (three warm, three cool), unsympathectomized limbs showed definite reductions of RHBF during smoking while the sixth showed no change. The contralateral (sympathectomized) limbs showed no change of RHBF in four instances and a diminution of RHBF definitely less than that of the sympathectomized side in two instances.

These studies show that tobacco smoking often reduces post-ischemia vasodilatation, and that this reduction is apparently mediated via the sympathetic nervous system.

Use of Indicator-Dilution Curves in the Diagnosis of Valvular Insufficiency. Edward Woodward, Jr., F. Henry Ellis, Jr., and Earl H. Wood,* Rochester, Minn.

Following rapid injection of an indicator into the vascular system, traversal of the resultant dye-blood mixture through a cardiac chamber, which is guarded by an incompetent valve or at which a left-to-right shunt occurs, results in a decrease in peak concentration, a prolongation of the disappearance slope, and a relative decrease in the appearance time of the indicator-dilution curves recorded down stream to the site of the abnormality. These parameters of the dilution curve also are affected by the cardiac output and central blood volume; hence, their use for detection of valvular insufficiency or a shunt requires means for compensating for variations in flow and volume in the individual patient. The DT/BT ratio, and the variance and disappearance of slope ratios of Korner and Shillingford have been used. The use of these and other parameters of dilution curves in distinguishing between patients with predominant stenosis or predominant insufficiency of the mitral valve has been investigated by a study of dilution curves recorded continuously at the radial artery after sudden single injections of T-1824 (7.5 to 15 mg.) into the pulmonary artery of 11 healthy persons and 30 patients with mitral valvular disease. Further, the relationship of mitral insufficiency to the quantity of dye rapidly appearing in the left atrium following left ventricular injection has been studied in 10 dogs before and after surgical production of insufficiency and in 20 patients with valvular disease.

A correlation was established between the degree of insufficiency and each of 12 parameters studied. None provided a completely uniform separation of patients with predominant stenosis and those with predominant insufficiency. However, frequencies of overlap as low as one in 30 establish the indicator-dilution method as a valuable adjunct to the differential diagnosis of mitral valvular disease.

The Elaboration by Tumor Cells of Lactic Dehydrogenase into the Bathing Fluids: In Vitro and In Vivo Studies. Felix Wróblewski and Alice E. Moore, New York, N. Y. (Introduced by Rulon W. Rawson).

It has been suggested that cancer cells originate from normal cells in two phases: (1) irreversible injury of respiration and (2) replacement of lost respiration energy by fermentation energy. This hypothesis is consistent

with the observations that some experimentally induced and clinical states of malignant neoplasia manifest increased serum lactic dehydrogenase activity.

In vitro tissue cultures of normal cells including amnion and fibroblast in Eagles medium were incubated at 37° C for ten days; similarly, tissue cultures of malignant neoplastic cells including Osgood J-111 leukemia, Osgood J-96 leukemia, HEP-2, HEP-1, and HeLa were incubated. The bathing fluid medium of each of the normal and cancerous tissue cultures was examined daily for lactic dehydrogenase activity. The enzyme activity of the media of normal cells remained the same or increased slightly during a ten-day period. The lactic dehydrogenase activity of the media of the cancerous cells increased progressively and reached values of as much as fifteen times the initial enzyme activity of the media.

Pleural and peritoneal effusions from patients with congestive heart failure, cirrhosis, tuberculosis and metastatic cancer were examined for lactic dehydrogenase activity. Simultaneously, the enzyme activity of the serum of the same individuals was determined. In most instances we have found that effusions containing malignant cells manifest lactic dehydrogenase activity greater than the enzyme activity of the serum obtained simultaneously from the same individual. In pleural and peritoneal fluids free of malignant cells we have found lactic dehydrogenase activities which were less than the respective serum lactic dehydrogenase.

It would appear that lactic dehydrogenase is elaborated into the bathing fluids by tumor cells. This characteristic of malignant cells *in vitro* and *in vivo* may serve to distinguish biochemically normal from cancerous cells.

Overproduction of Uric Acid as the Cause of Hyperuricemia in Primary Gout. JAMES B. WYNGAARDEN, Durham, N. C. (Introduced by Grace P. Kerby).

Stetten and associates demonstrated that certain gouty subjects incorporated excessive quantities of glycine-N^{15} into urinary urate, whereas certain others did not. These studies have been repeated, employing a *tracer* dose of glycine-1-C^{14} in place of glycine-N^{15}. Six consecutive gouty subjects have shown abnormally high concentrations and abnormally great cumulative incorporations of C^{14} in urinary urate. Five of these subjects had normal urinary urate excretions; in one there was asymptomatic hyperuricemia. Degradation studies of urinary urate have shown that glycine-1-C^{14} has labeled urate specifically (97 per cent) in C-4. These studies demonstrate that overproduction of urate is the metabolic defect of primary gout. Studies of the labeling patterns of urinary hypoxanthine, xanthine, adenine, guanine and 7-methylguanine suggest that synthesis of urate in control, gouty and leukemic subjects involves two types of pathway, the one an early cleavage of newly formed nucleotides, the other the catabolism of nucleic acids. The data suggest that the first type of pathway participates in the excessive synthesis of labeled urate in primary gout, the second in secondary gout.

These studies permit formulation of a working hypothesis regarding the metabolic defect of primary gout: Normally the quantity of purine nucleotides synthesized somewhat exceeds that required for nucleic acid synthesis. Excess nucleotides are cleaved, and the purine moieties oxidized to urate. The rate of purine nucleotide synthesis is regulated by a feedback system (as recently demonstrated in *E. coli* for pyrimidine synthesis). In primary gout this feedback mechanism fails to operate effectively. This defect permits excessive production of purine nucleotides, which are promptly cleaved and oxidized, resulting in overproduction of urate by a direct pathway not involving nucleic acids as intermediates. A lesion of the feedback mechanism itself, or an excess activity of the enzymes of nucleotide cleavage, or of base oxidation, is a possible explanation. This hypothesis is consistent with existing information on purine metabolism in normal and gouty persons.

Increased Permeability of Muscle Membranes Produced by Insulin. KENNETH L. ZIERLER,* Baltimore, Md.

There are two major theories on the mechanism of insulin action, one holding that it acts at an enzymic level, the other that it accelerates movement of glucose across cell membranes. The latter hypothesis may imply that insulin acts on the membrane itself, altering permeability.

When intact rat skeletal muscle is incubated intracellular material as large as proteins diffuses from tissue to solution. Aldolase is one such substance and rate of diffusion of aldolase may be used as an index of muscle membrane permeability.

In 17 pairs of muscles in Krebs-Ringer-HCO_3-glucose, aldolase diffusion was increased by 40 per cent by addition of insulin. When the membrane was depolarized by a bathing solution rich in potassium, aldolase diffusion was accelerated greatly. In 30 pairs of muscles in this solution insulin increased aldolase diffusion by an additional 20 per cent, a value significant at $P = 0.001$. It is concluded that in isolated muscle under these conditions insulin increased membrane permeability.

Effect of Naphthalene Derivatives on Glutathione Metabolism of Erythrocytes from Patients with Naphthalene Hemolytic Anemia. WILLIAM H. ZINKHAM and BARTON CHILDS, Baltimore, Md. (Introduced by C. Lockard Conley).

Hemolytic anemias due to primaquine and certain other drugs have been shown to occur in people with an intrinsic abnormality of the erythrocyte. This abnormality is characterized by relative deficiency of reduced glutathione (GSH) and marked decrease of GSH when erythrocytes are incubated with acetylphenylhydrazine (APH). These erythrocytes are not susceptible to acceleration by naphthalene of taurocholate hemolysis.

Severe hemolytic anemia has occasionally been observed in people exposed to naphthalene ($C_{10}H_8$). To determine the nature of this process, GSH levels before

and after incubation with APH, naphthalene, and naphthalene metabolites were measured in erythrocytes from 4 negro patients who developed fulminating hemolytic anemia following exposure to naphthalene moth balls. There was marked reduction in GSH after incubation with APH. No significant decrease of GSH was observed when erythrocytes were incubated with naphthalene. Four naphthalene derivatives: alpha- and beta-naphthol and alpha- and beta-naphthoquinone, produced a marked fall in GSH. This effect was observed in concentrations which failed to reduce GSH of normal erythrocytes. Menadione sodium bisulfite (Hykinone®), a derivative of alpha-naphthoquinone, was equally effective in decreasing GSH. These experiments indicate that naphthalene metabolites rather than naphthalene are the active hemolytic agents.

This abnormality in GSH metabolism was also observed in a number of relatives of these patients. The distribution of the abnormality within these families demonstrates that this characteristic is genetically determined, and is probably due to a dominant mutant gene. It is noteworthy that in the cases studied, as well as bulk of cases reported in the literature, the patients are negroes. These observations suggest that susceptibility to naphthalene hemolytic anemia may be restricted to certain members of the population.

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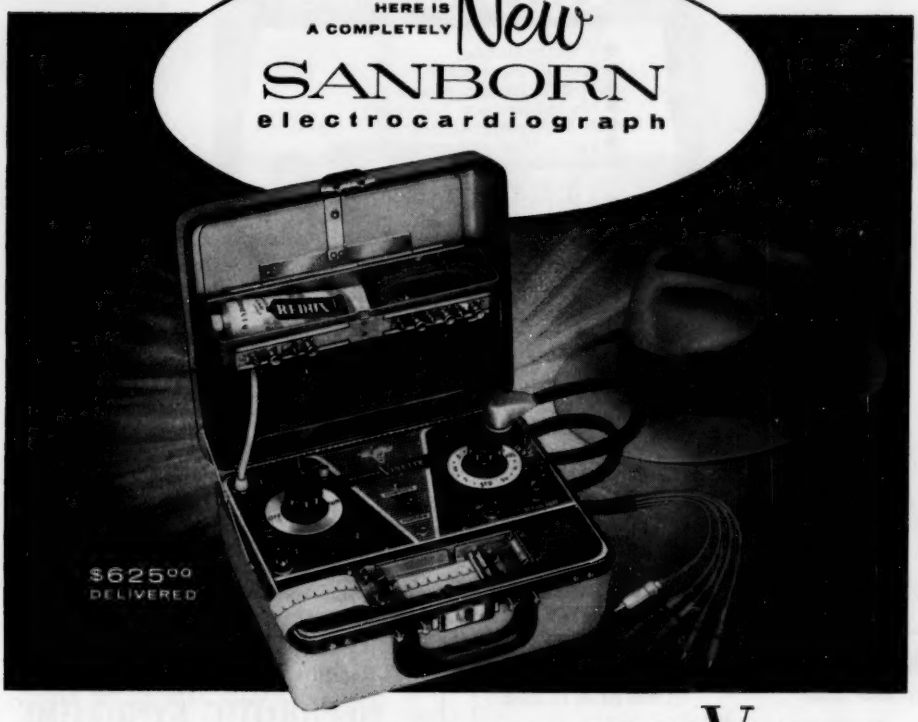

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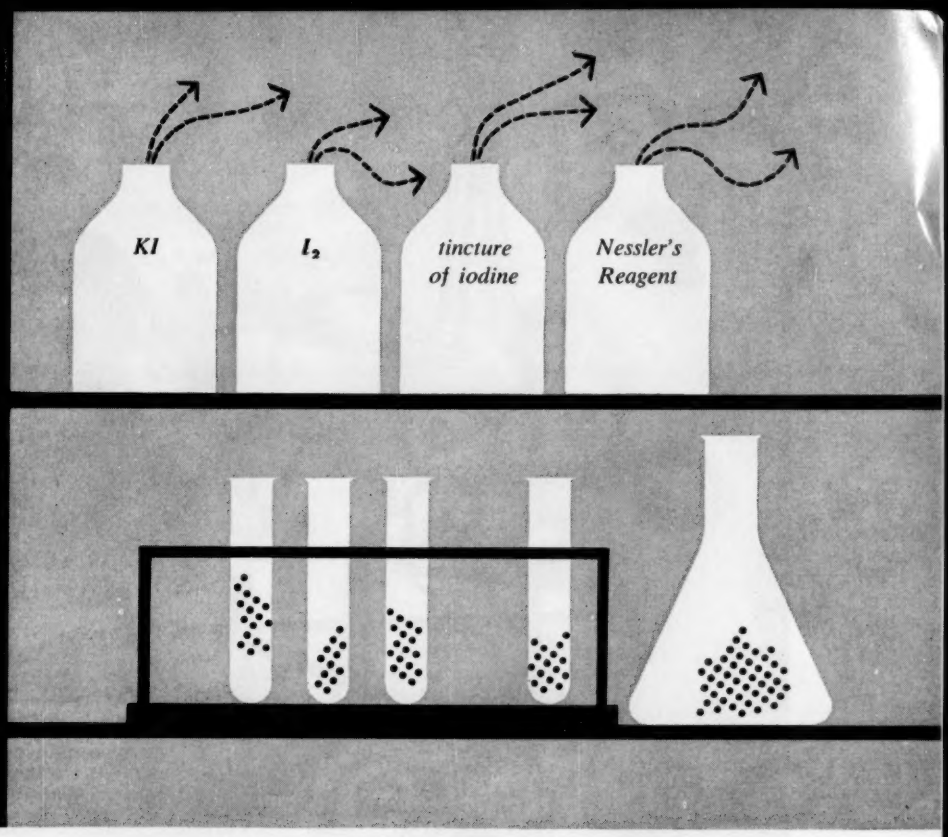

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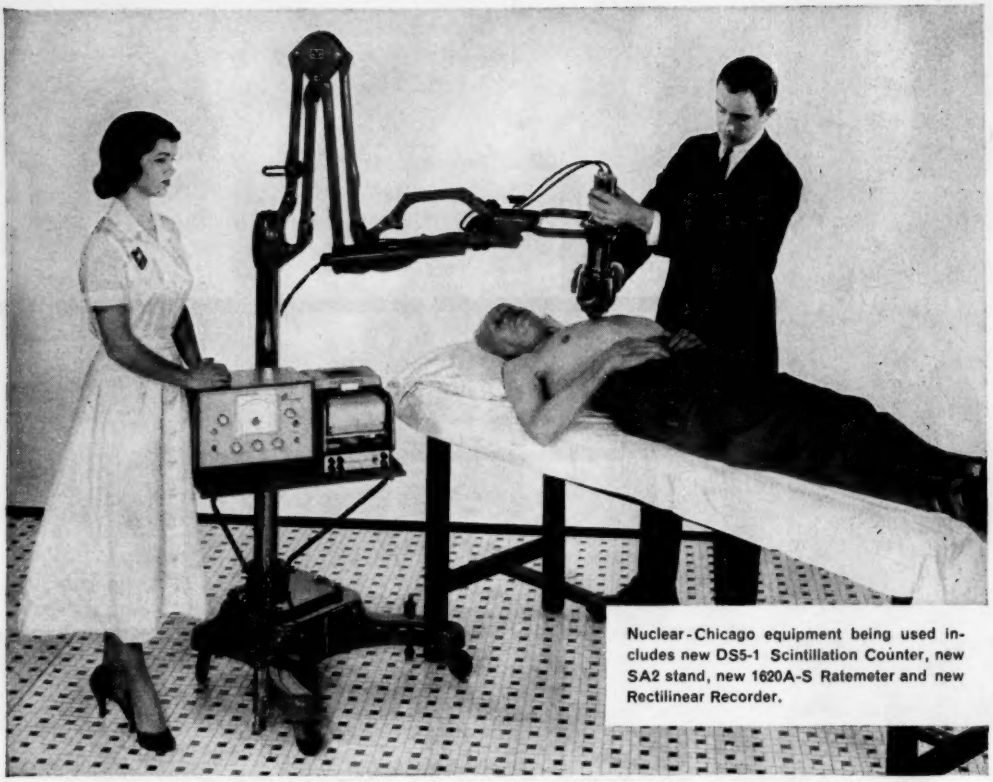

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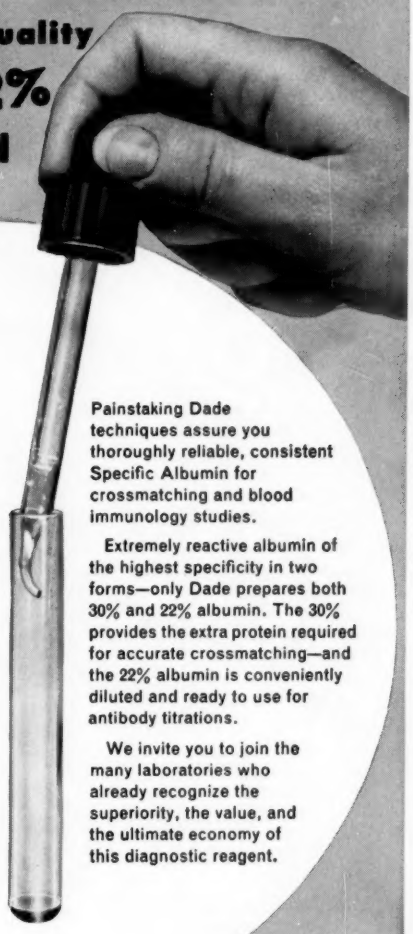

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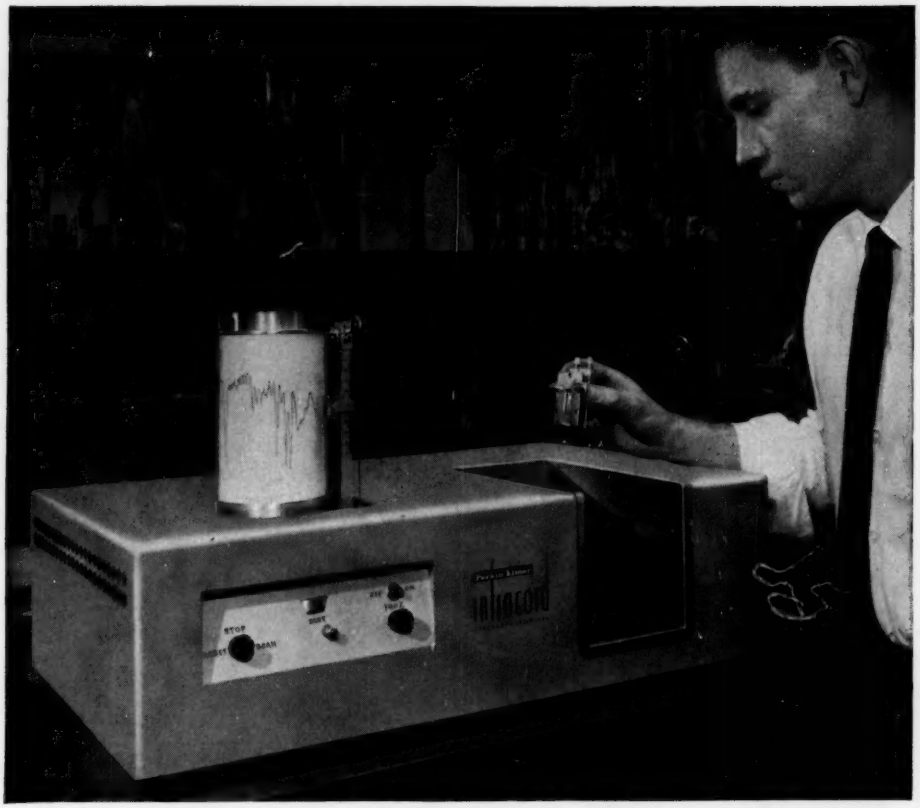

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PART II

The Journal of Clinical Investigation

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METABOLIC STUDIES IN PARALYTIC ACUTE ANTERIOR POLIOMYELITIS [1]

I. ALTERATIONS IN NITROGEN AND CREATINE METABOLISM.

II. ALTERATIONS IN CALCIUM AND PHOSPHORUS METABOLISM.

III. METABOLIC AND CIRCULATORY EFFECTS OF THE SLOWLY OSCILLATING BED.

IV. EFFECTS OF TESTOSTERONE PROPIONATE AND ESTRADIOL BENZOATE ON CALCIUM, PHOSPHORUS, NITROGEN, CREATINE AND ELECTROLYTE METABOLISM.

APPENDIX A: TABLES OF METABOLIC BALANCE DATA.

APPENDIX B: CASE SUMMARIES OF ELEVEN PATIENTS WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS.

By G. DONALD WHEDON [2] AND EPHRAIM SHORR †

WITH THE TECHNICAL ASSISTANCE

OF VINCENT TOSCANI AND ESTELLE STEVENS

(From the Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College, and the New York Hospital, New York, N. Y.)

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

[2] Present Address: National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

† Died, January 6, 1956.

METABOLIC STUDIES IN PARALYTIC ACUTE ANTERIOR POLIOMYELITIS. I. ALTERATIONS IN NITROGEN AND CREATINE METABOLISM [1]

By G. DONALD WHEDON [2] AND EPHRAIM SHORR †
WITH THE TECHNICAL ASSISTANCE
OF VINCENT TOSCANI AND ESTELLE STEVENS

(From the Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College, and the New York Hospital, New York, N. Y.)

(Submitted for publication July 27, 1955; accepted November 26, 1956)

For hundreds of years acute anterior poliomyelitis has been regarded as involving exclusively the motor nerves and the muscles served by them. Prolonged after-effects on muscles have called attention to chronic features of this acute infectious disease, but serious and persistent involvement of systems other than the muscular, such as the skeletal, urinary or respiratory, has been barely recognized.

The studies to be reported show that infection with the virus of poliomyelitis, while undoubtedly exercising a primary effect on motor and other nerve cells of the spinal cord and brain, may have pronounced secondary effects upon many systems and particularly upon muscle and skeletal metabolism.

The major objective of these studies was to outline the metabolic and physiological features of the natural course of paralytic acute anterior poliomyelitis during acute, convalescent and chronic phases. The most important changes with respect to nitrogen, creatine, calcium and phosphorus are presented in the first two papers of this series of four; disturbances in the metabolism of electrolytes are given in the fourth paper. Since patients with this disease could not be studied untreated, the natural course is defined as evolution of the illness during treatment with standard modern methods of physical therapy. Using these observations of the natural course as a base-line, assessment was made of metabolic and circulatory influences of the slowly oscillating (Sanders) bed (1), described in the third paper, and of the effects of androgenic and estrogenic hormones, reported in the fourth paper.

The investigation was initiated as a result of previous observations of the effects of immobilization (2) in four normal young male subjects which had revealed significant losses of nitrogen, calcium and other minerals, disturbance in creatine metabolism and in peripheral circulation, as well as losses of muscle mass and strength. In view of the substantial derangements resulting from immobilization *per se* in healthy individuals, it seemed desirable to ascertain to what extent metabolic alterations might occur in a disease such as acute anterior poliomyelitis when associated with prolonged and extreme immobilization.

PREVIOUS OBSERVATIONS OF METABOLISM IN POLIOMYELITIS

Few observations have been made revealing abnormal metabolism in poliomyelitis. Creatinuria accompanying the chronic stage of acute anterior poliomyelitis was first described by Levene and Kristeller (3) in 1909. Other observers have also noted diminished urinary creatinine (4-10) and diminished retention of exogenous creatine (6-10) during the convalescent and chronic phases. A few measurements of creatinuria during the early weeks of the disease (11-13) have been made but revealed no consistent pattern of excretion. In these latter studies, the value of the creatine determinations is suspect, because extreme variations in 24-hour creatinine excretion in individual patients indicated incomplete urine collections.

The relationship of creatinuria to virus infection has been investigated in animals by Gifford and Dalldorf (14), who detected creatinuria in suckling mice experimentally infected with Coxsackie virus.

Whether correlation can be shown in patients between the level of creatinuria and extent of muscle paralysis is unsettled. In comparable observations, Wang (11) after study of eight cases and Brøchner-Mortenson (12) from twelve cases came to opposite conclusions.

Various isolated studies suggesting abnormal nitrogen metabolism in poliomyelitis have been carried out from

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

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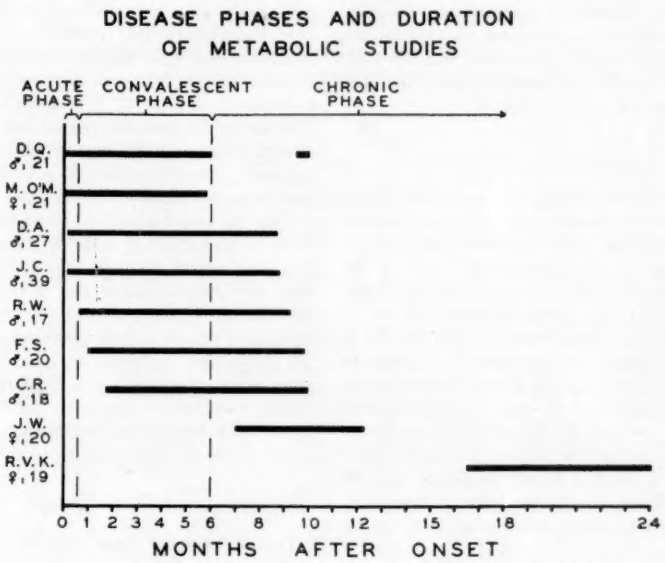

FIG. 1. THE PHASES OF ACUTE ANTERIOR POLIOMYELITIS DURING WHICH METABOLIC STUDIES WERE CARRIED OUT IN NINE PATIENTS, AND THE DURATION OF THESE STUDIES IN EACH CASE

which few conclusions can be drawn. Nitrogen balance has been carefully though briefly measured by only one investigator, Bürger (4) in 1919, who found a small negative balance lasting a few days in one of two patients studied several weeks after onset of illness. Data on urinary nitrogen excretion have been presented by Bower, Morgan, and Chaney (15) on 17 patients observed for periods up to three months after onset. In these patients, nearly all of whom required the aid of a respirator, large amounts of nitrogen were excreted in association with a fall in serum albumin and rise in serum globulin. Bower, Eaton, Chudnoff, Affeldt, and Chaney (16) had previously reported similar alterations in serum albumin and globulin in severely involved patients during the acute phase of poliomyelitis. Routh and Paul (17) confirmed these findings by electrophoretic analysis, but Steigman, Brodsky, and Stephens (18) in 125 patients could find no decline in serum albumin during the acute phase. This was the state of the literature at the time these papers were being written; several reports have been included (12-18) which were published while we were engaged in these studies.

GENERAL PLAN

Eleven patients were studied, all with marked and wide-spread muscle weakness and paralysis. Nine were studied on the Metabolism Ward of the Russell Sage Institute of Pathology and the New York Hospital for periods ranging from five to nine months. Figure 1 indicates for each of these nine patients the duration of study and phase of disease during which study was carried out. In four of the nine, investigation was begun early in the acute phase of the disease. Two patients were observed for brief periods on the Infectious Disease Ward only, under less rigidly controllable conditions, and are reported only in the third paper, on the effects of the oscillating bed.

Of the nine patients reported in this paper, one received no treatment other than generally accepted standard medical and physical therapy (to be described below) throughout the six months he was observed, beginning on the day of onset of paralysis. The other patients also received conventional therapy but in addition, interrupting observation of the natural course of their disease, were either placed on a slowly oscillating Sanders bed or given androgens or estrogens for a few weeks at a time on one or more occasions. The data obtained during and for two to four weeks after the intervals in which these special procedures were employed have been omitted from the present report in order to provide as uncomplicated as possible a picture of the natural course, and have been reserved for subsequent papers in this series. Exclusion of these data is responsible for the interrupted nature of a number of the graphs in this paper. Consecutive metabolic data, including periods on and following special procedures, are given in tables in Appendix A.

The patients ranged in age from 17 to 39 years. Three (M.O'M., J.W., and R.V.K.) of the nine were females. Muscle paresis was extensive in all, seven of the nine having all four limbs involved; in two (D.Q. and J. C.), the upper extremities were unaffected. Two (C.R. and R.V.K.) required tank respirators for a few weeks prior

to entering this study. Extent of muscular paralysis was estimated from reduction in urinary creatinine and ranged from 30 to 80 per cent, with most patients showing a 40 per cent to 60 per cent loss of muscle mass. Detailed case summaries are given in Appendix B.

PROCEDURE

The four patients whose studies were initiated during the acute phase were first observed on the Infectious Disease Ward. Daily 24-hour urine collections were begun on the third day after onset of illness with D.Q., on the fourth day after onset with M.O'M., and on the sixth day with D.A. and J. C. Onset of illness was taken as the first day on which the temperature was elevated to 100° F or above; this was usually two or more days prior to hospital admission. In the case of D.Q., we began urine collections on the morning of the day muscle weakness was first noted; in M.O'M., D. A., and J. C. urine collections were begun on the first, second and fourth days, respectively, following onset of muscle weakness. On the Infectious Disease Ward, record was made of daily fluid and food intake, and the patients were weighed at weekly intervals.

The four patients were transferred to the Metabolism Ward 14 to 21 days after onset of illness, 8 to 10 days after the temperature had returned to normal levels. As soon as each patient could eat a constant diet, collection and analysis of stool were begun, in addition to those of urine, so that balances could be calculated. Rigid precautions against contagion were observed by the nursing staff in handling stool specimens. Drying the stools at 100° C for 24 to 48 hours over a steam bath was considered adequate to inactivate the virus prior to chemical analysis.

The five remaining patients in the group had been cared for in other hospitals during the acute phase of their illness and, when transferred to the New York Hospital, were admitted directly to the Metabolism Ward. Metabolic study of three of these patients was begun during the convalescent phase of their disease, the observation of R.W. beginning on the 19th day after onset of illness, that of F.S. on the 30th day and that of C.R. on the 49th day. Studies on J.W. and R.V.K. were begun during the 7th and 17th months, respectively, after onset of illness.

PATIENTS' CARE AND THERAPY

Management of patients during the acute phase involved not only care of muscles but attention to comfort, respiration, sphincter control and fluid balance. Urinary infections in two patients following catheterization responded promptly to brief courses of sulfadiazine.

Hot pack therapy to the entire back and extremities was instituted upon admission and was administered for 3 to 8 hours daily for two and one-half months; in one patient who had prolonged muscle stiffness, C.R., hot packs were continued for four and one-half months. In some instances packs were supplemented with hot tubs. Passive motion of extremities and muscle stretching twice daily were begun within the first ten days, shortly after temperature had returned to normal. Muscle re-education was generally added during the third week. Initiated in bed, this procedure was later carried out in a therapeutic pool at 92° F and still later in a gymnasium. Therapy in the pool was started two to two and one-half months after onset of illness or approximately at the time hot packs were discontinued, and was gradually lengthened from 15 minutes to 45 minutes once or twice daily.

Time of mobilization to wheel-chair and, when possible, to standing and walking varied widely. Patients D.Q., M.O'M., D.A., J.C., and R.W. began sitting in a chair two and one-half to four months and walking three and one-half to seven months after onset. F.S. and C.R., with more severe muscle involvement, began sitting near the end of their metabolic study, approximately 9 months after onset. J.W. and R.V.K., who were studied only during the chronic phase, sat up for one and one-half hours daily throughout the study. Further details are given in the patients' case summaries, Appendix B.

DIETS

During the acute phase, appetite was poor and food intake low for a few days, but both were variably restored within the first two weeks. During this phase food intake was recorded and calculated. Only the intake of milk was regulated, at three glasses daily, to limit calcium intake to 1.0 gram. Fluid intake was maintained at more than 2000 ml. daily.

When the patients were transferred or admitted directly to the Metabolism Ward, they began to receive constant dietary intakes (Table I). For each diet there were three or four daily menus rotated in succession which were virtually identical in caloric value and protein and mineral content; one menu was creatine-creatinine free (containing no meat or fish). When necessitated by a markedly altered appetite demand or an undesirable trend in body weight, a change was made in the diet to a new constant caloric level without changing protein and mineral intake. Protein intake was set at levels of 1.0 to 1.4 grams per kilogram of pre-illness body weight. Mineral constituents were also set at moderate but adequate levels. Sodium intake was kept constant at 4.0 grams (sodium chloride, 10.25 gm.) per day in five studies by giving the patients weghed amounts of extra sodium chloride to be sprinkled over the food; the amounts added depended on the sodium values of the diets as determined by analysis.

Calculated values for caloric and mineral content of foods were obtained from standard tables (19–21). Sodium and potassium values were obtained from Mead Johnson reports (22, 23).

Twenty-four analyses of diets were made during the study, at least one of each menu used at each caloric level, amounting to a little more than 1 per cent of the approximately 1900 calculated and weighed diets served during the entire investigation. Average percentage of calculated values found by analysis was 103.1 per cent for nitrogen, 101.2 per cent for calcium, 95.5 per cent for

TABLE I

Summary of calculated constant dietary intakes, convalescent and chronic phases of paralytic acute anterior poliomyelitis

Patient	Number of six-day periods on diet	Interval on diet, days after onset	Calories	CHO	Fat	Protein	N	Ca	P	K	Na
				gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
D. Q.	4	32–56	2,020	199	100	80	12.82	0.920	1.416	2.80	4.00
	20	57–176	2,408	297	100	80	12.82	0.921	1.462	3.01	
	2	282–297	2,408	297	100	80	12.82	0.921	1.462	3.01	
M. O'M.	24	25–173	1,801	200	80	70	11.28	0.921	1.354	2.65	
D. A.	21	17–146	2,800	353	114	90	14.37	0.920	1.638	3.50	4.00
	19	147–261	2,000	209	89	90	14.42	0.920	1.545	2.88	4.00
J. C.	16	23–123	2,397	274	109	80	12.78	0.919	1.434	3.07	
	23	124–262	2,797	349	120	80	12.80	0.921	1.424	4.14	
R. W.	12	22–93	2,002	202	97	80	12.80	0.920	1.352	2.78	4.00
	13	94–171	2,398	274	109	80	12.80	0.920	1.434	3.08	4.00
	18	172–280	2,801	333	128	80	12.78	0.920	1.453	3.54	4.00
F. S.	43	31–294	2,798	351	115	90	14.40	0.922	1.659	4.09	4.00
C. R.	40	50–300	1,997	199	98	80	12.82	0.921	1.379	2.66	4.00
J. W.	23	212–369	1,803	222	66	80	12.84	1.005	1.709	2.75	
R. V. K.	25	503–656	1,595	208	59	58	9.36	0.888	1.329	2.81	
	13	657–735	1,938	233	78	76	12.16	1.478	1.794	3.52	

phosphorus, 98.7 per cent for sodium and 100.4 per cent for potassium. However, appreciable day to day variation, amounting to ± 6 to 9 per cent, is indicated by the standard deviations of analyzed from calculated values (± 0.82 gm. for nitrogen, ± 0.061 gm. for calcium, ± 0.131 gm. for phosphorus, ± 0.163 gm. for sodium and ± 0.302 gm. for potassium).

METHODS

Ward routine and methods employed on the Metabolism Ward have been previously reported (24). Procedures are similar to those of Sampson, Sprague, and Wollaeger (25) and Reifenstein, Albright, and Wells (26).

Urinary nitrogen, creatine, creatinine, pH and specific gravity were determined on 24-hour urinary specimens. Three-day pooled specimens made up from 10 per cent aliquots of the 24-hour collections were used for the analyses for urinary glycocyamine, calcium, phosphorus, citric acid, and sulphur. Analyses for 17-ketosteroids were carried out during the early weeks on three-day and later on six-day pooled specimens. Pooled specimens were preserved for many months for check analyses by the addition of 2 ml. conc. HCl per 100 ml. of specimen. Sodium, potassium, and chloride analyses were performed on unacidified refrigerated three-day pooled specimens.

Balance studies were carried out in six-day periods. Fecal nitrogen, calcium, phosphorus, sodium, and potassium were determined by chemical analysis. Methods of stool marking, collection and preparation for analysis have been reported elsewhere (27). Diets were analyzed *in toto* (rather than by separate analyses of individual foods), a one-half aliquot of a day's menu being weighed out, mixed together, ground and then prepared for analysis in the same manner as in the preparation of a stool for analysis.

Nitrogen in urine and stool was analyzed by a modification of the Kjeldahl method (28). Urinary total sulphate reported as sulphur was determined by the method of Folin (29), urinary creatine by the Benedict modification of Folin's method (30) (which converts creatine to creatinine) and creatinine by the method of Folin (31). Urinary 17-ketosteroids were determined by a modification of the method of Callow, Callow, and Emmens (32) previously described (2); normal values in our laboratory are 6 to 12 mgm. per day for women and 8 to 14 mgm. per day for men.

Creatine tolerance tests were performed as follows: patients were given a creatine-creatinine-free menu for four successive days. Urinary creatine excretion on the second day was taken as the control value. On the third day 1.32 gm. of creatine hydrate powder in capsules was given. Calculation of creatine tolerance, or percentage of fed creatine retained, was based on the total excretion of creatine of the day of and the day following administration of the test dose, from which two-day total a figure was subtracted equal to twice the control day excretion. Minimal normal value for creatine tolerance, or retention, was regarded as 70 per cent with a test dose of 1.32 gm. creatine hydrate.

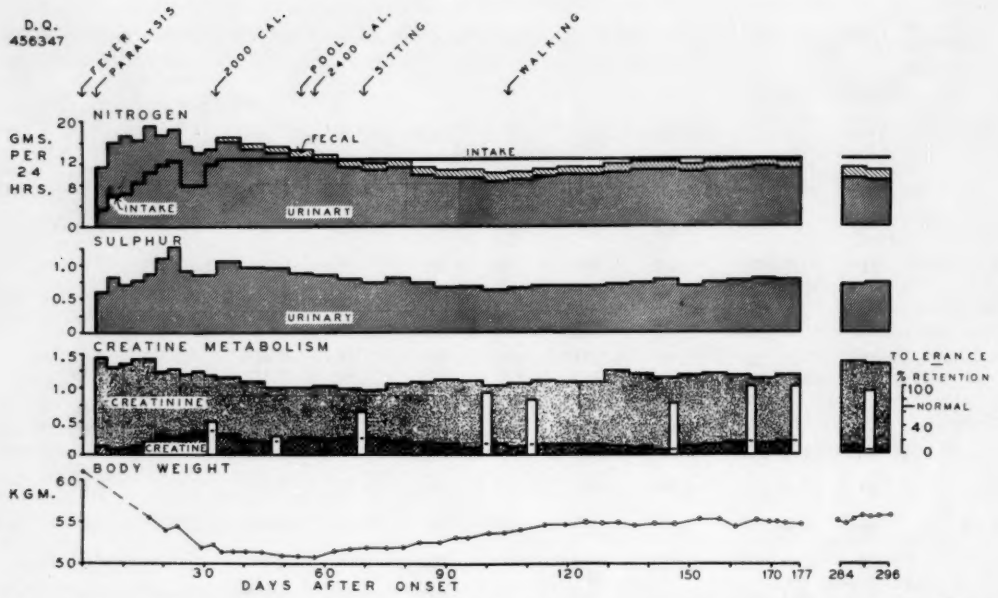

FIG. 2. NITROGEN BALANCE, URINARY SULPHUR, CREATININE AND CREATINE, CREATINE TOLERANCE AND BODY WEIGHT IN PATIENT D.Q., 21-YEAR-OLD MALE, DURING FIRST SIX AND TENTH MONTHS AFTER ONSET

Patient transferred to the Metabolism Ward on 14th day after onset. The balance data in this and in subsequent graphs are charted with the intake and output both plotted upward from the base-line and urinary output on the bottom in order to portray clearly changes in urinary excretion as well as changes in balance. In this and in the following graphs creatine tolerance is indicated by open bars in the creatine metabolism at the points when the tests were made.

In determination of urinary glycocyamine, arginase was prepared by the method of Hunter and Downs (33). The analytical procedure for glycocyamine was that of Hoberman (34) with substitution of the quantities of reagents used in colorimetry by Dubnoff and Borsook (35).

Serum total proteins were determined by a slight modification of the Kjeldahl method for urine (28), *viz.*, 25 ml. of saline were added to 1 ml. of serum in the Kjeldahl flask to prevent frothing.

Girth of thighs and calves was measured at standard distances from the internal malleolus with the patient supine, generally every four to eight days. A device previously described (2) involving use of a wide metal band encircling the leg under constant and equal tension permitted measurements more accurate than those with a narrow steel measuring tape.

Muscle strength was measured in five patients every one to three weeks on an ergometer previously described (2). Muscle groups tested were the biceps, anterior tibial and gastrocnemius-soleus.

Muscle testing by Lovett technique (36) was carried out at intervals of 4 to 6 weeks by the physical therapist.

RESULTS

A. *Acute and early convalescent phases*

Patterns of altered metabolism can be more easily described if consideration is not limited to the acute phase (first ten days to two weeks after onset of illness) but includes the data of the *first month*. Description is based on the data of patients D.Q., M.O'M., D.A., and J.C. (Figures 2 to 5).

The clinical course of these four patients during the acute phase was usual for acute anterior poliomyelitis. None had prodromata. All four had a single-humped fever curve, lasting six to nine days, accompanied by headache, malaise, muscle tenderness, and mild gastro-intestinal symptoms. Paralysis was noted on the third, third, fourth, and second day, respectively, after the onset of fever.

1. *Nitrogen.* During the first two weeks after onset of illness urinary nitrogen excretion progres-

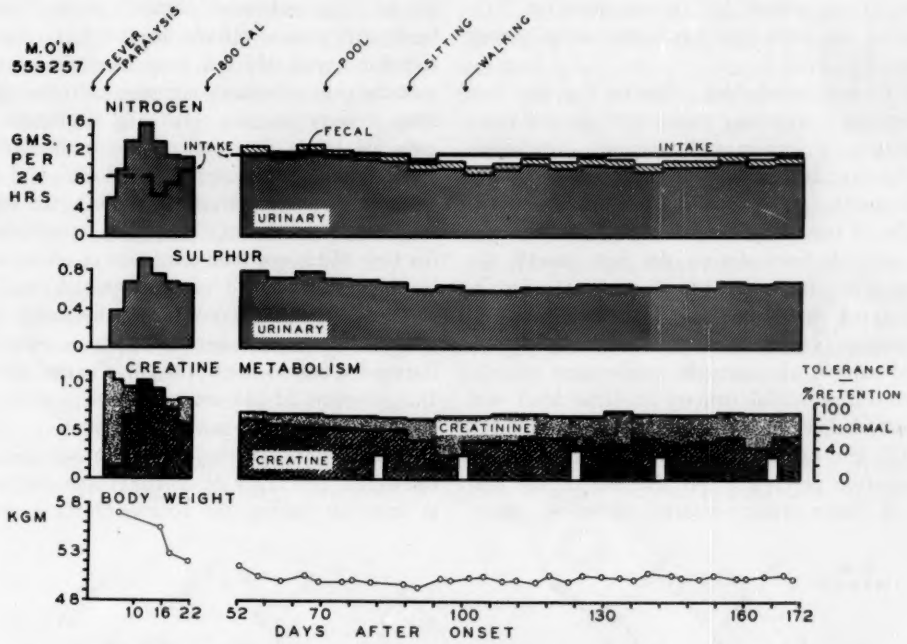

FIG. 3. NITROGEN BALANCE, URINARY SULPHUR, CREATININE AND CREATINE, CREATINE TOLERANCE AND BODY WEIGHT IN PATIENT M.O'M., 21-YEAR-OLD FEMALE, DURING FIRST THREE WEEKS AND THIRD THROUGH SIXTH MONTHS AFTER ONSET

Patient transferred to Metabolism Ward on 13th day after onset. The interruption in this graph and those in subsequent graphs result from the exclusion of data for the periods *during* and for from two to four weeks *following* the administration of special procedures (*see text*).

sively increased. Maximal excretion occurred uniformly during the first half of the third week, the 16th day after onset being the mean, more than a week after fever had subsided. Late in the third week nitrogen excretion began to fall. No indication of increase in fecal nitrogen appeared during the lengthy period of marked negative nitrogen balance.

At its peak urinary nitrogen ranged from 17.5 to 29.8 grams per day among the four patients, the mean for the four being 23.4 grams per day. From stool nitrogen determinations begun shortly thereafter, nitrogen balance at the time of peak urinary nitrogen excretion may be estimated as ranging from − 10.1 to − 17.3 grams per day, the mean being −12.2 grams per day; this negative nitrogen balance is equivalent to a *daily* loss of 400 grams, or approximately one pound, of muscle protoplasm. The degree of nitrogen depletion was highly significant since at this time nitrogen intake was greater than 10 grams per day in three of the four patients. Total *measured* N loss during the first 22 days, summed from the data for only those days on which intake and output were known exactly, ranged from 89 to 185 grams among the four patients, the mean being 139.2 grams. While actual total N loss during the first three weeks would be somewhat more than this, the mean *measured* N loss alone during this period was equivalent to more than 4½ Kg. of protoplasm.

2. *Sulphur.* Urinary sulphur excretion gradually increased during the first two weeks, reaching a peak which usually coincided closely in time with that of nitrogen. A noticeable lag, however, in the amounts of sulphur being excreted in relation to nitrogen, was indicated by urinary nitrogen: sulphur ratios. During the second and third weeks these ratios were more than 17:1 (Figure 6), definitely above the ratio of nitrogen to sulphur in muscle tissue (37, 38). The degree of elevation

varied; in one patient, J.C., it was slight; in D.Q. excessive, the ratio reaching in the second week a value of 22.7 : 1.

3. *Creatine metabolism.* During the first two weeks urinary *creatinine* excretion fluctuated more than is usually seen in well-controlled metabolism experiments; later a variable fall reflected loss of muscle mass. Creatinine index (urinary creatinine per 24 hrs./body weight on day of determination) also declined during the first month, the reduction ranging from 21 to 56 per cent and averaging 36 per cent.

Excretion of urinary *creatine* followed a characteristic pattern when muscle involvement was extensive. The initial urinary creatine level was generally below the normal upper limit of 0.100 to 0.125 gm. per day for male patients not on a creatine-free diet (2). At the end of the first week of illness urinary creatine increased, gradually in D.Q., and rather abruptly in the three patients with greater muscle involvement. The rise ended in a peak which in three instances coincided with the peak of urinary nitrogen excretion; thereafter urinary creatine gradually declined. The maximal levels of creatinuria of patients D.A., J. C., and M.O'M. were 1.780, 1.605, and 1.080 gm. per day, respectively. In D.Q. the highest single day's excretion, 0.365 gm., coincided with the time of his maximal excretion of nitrogen, but his highest sustained elevation which was at a moderate level, occurred during the fourth week.

Only two tests of creatine tolerance were made during the first month of illness. On the 25th day, D.A. showed 52 per cent retention; on the 31st day D.Q. showed 49 per cent retention.

Urinary glycocyamine (guanidoacetic acid), the immediate precursor of creatine, was determined at intervals during the course of these studies.

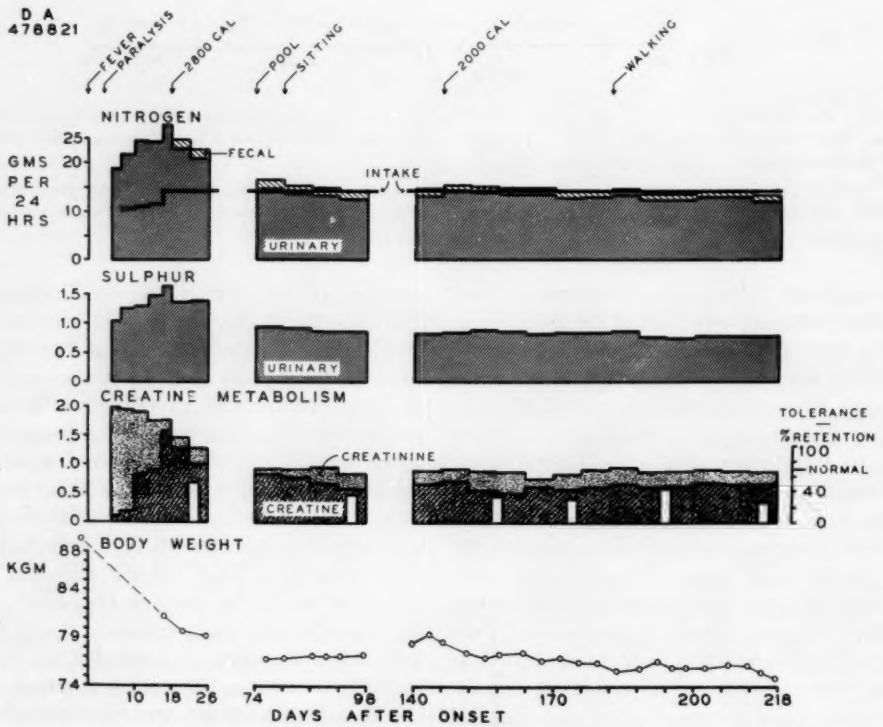

FIG. 4. NITROGEN BALANCE, URINARY SULPHUR, CREATININE AND CREATINE, CREATINE TOLERANCE AND BODY WEIGHT IN PATIENT D.A., 27-YEAR-OLD MALE, DURING FIRST, THIRD, AND FIFTH THROUGH SEVENTH MONTHS AFTER ONSET

Patient transferred to Metabolism Ward on 16th day after onset.

The few analyses revealed considerable variation but a statistically significant difference (difference between means more than 2 S. D.) in three of the four patients between excretion during the first three weeks and later levels. Mean early values and mean convalescent phase values, respectively, in mgm. per day were as follows: D.Q., 110, 80; M.O'M., 135, 89; D.A., 130, 94; and J.C. (difference not significant), 142, 122. None was above the normal limits of 30 to 150 mgm. per day of Sims and Seldin (39).

4. *17-Ketosteroids.* Of the four patients observed during the acute phase, one, D.Q., showed a distinct fall in urinary 17-ketosteroid excretion. His initial value on the 3rd to 5th day after onset was 5.5 mgm. per day. From then until six weeks after onset his 17-ketosteroid excretion ranged between 1.9 and 4.9 mgm. per day; thereafter, it fluctuated between 5.3 and 7.5 mgm. per day. Patient J.C.'s initial value was 7.4 mgm. per day; one 3-day pool midway in the second week was 5.9 mgm. per day, but thereafter the values ranged between 6.5 and 9.4 mgm. per day. The 17-ketosteroid excretion of patients M.O'M. and D.A. ranged between 7.6 and 9.9 and between 8.8 and 12.2 mgm. per day, respectively, throughout their stay.

5. *Body weight.* During the first three weeks loss of body weight was precipitate, averaging 0.4 Kg. per day. Caloric intake was generally variable during the first two weeks but could be considered low only during the febrile first week.

B. *Convalescent phase*

Definition of the duration of the convalescent phase in acute anterior poliomyelitis depends on

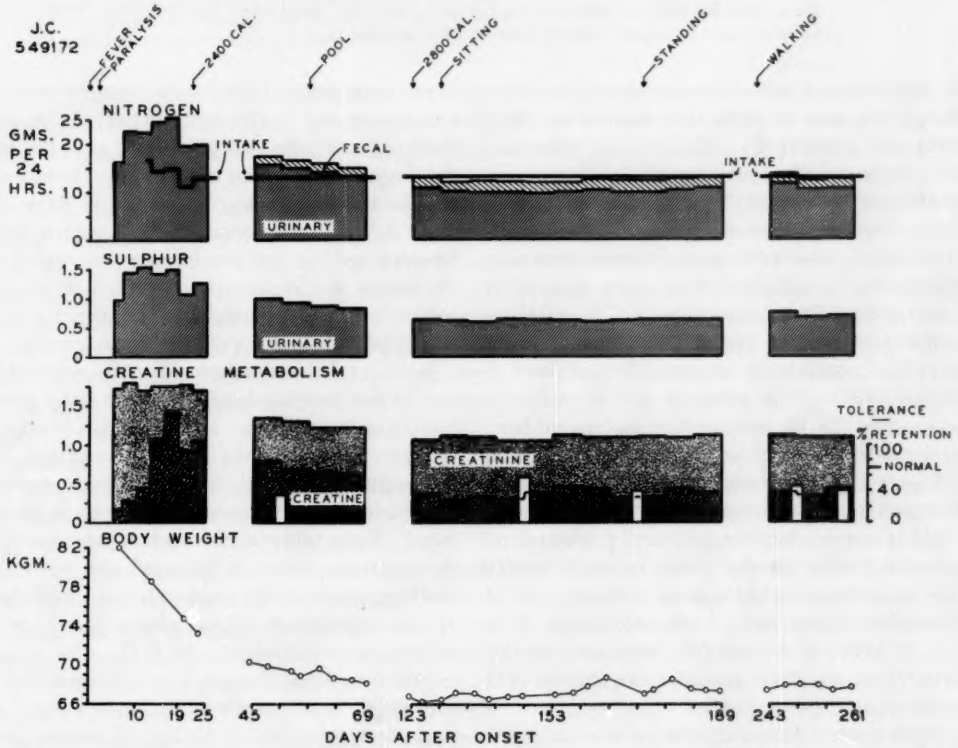

FIG. 5. NITROGEN BALANCE, URINARY SULPHUR, CREATININE AND CREATINE, CREATINE TOLERANCE AND BODY WEIGHT IN PATIENT J.C., 39-YEAR-OLD MALE, DURING THE FIRST, SECOND, FIFTH, SIXTH AND NINTH MONTHS AFTER ONSET

Patient transferred to Metabolism Ward on 22nd day after onset.

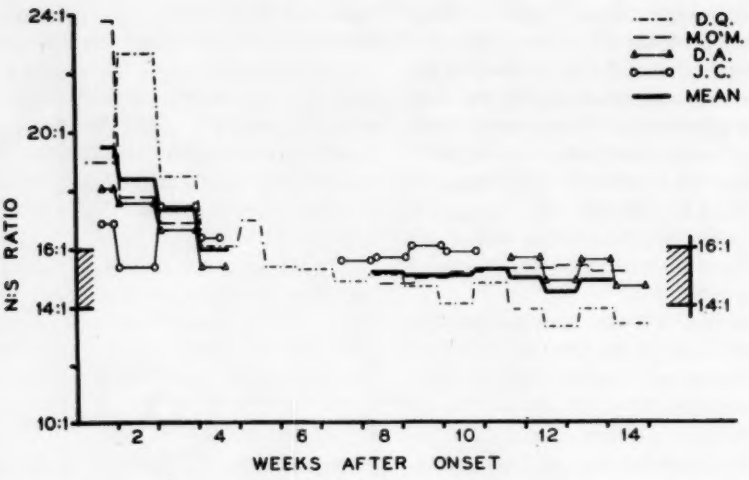

FIG. 6. RATIOS OF URINARY NITROGEN TO URINARY SULPHUR EXCRETION IN THE FOUR PATIENTS STUDIED DURING THE ACUTE AS WELL AS CONVALESCENT PHASE

Mean ratio for the four patients is indicated by the solid black line. Normally the urinary nitrogen to sulphur ratio is between 14:1 and 16:1.

the aspect of the disease taken as criterion. Morphologic recovery of nerve cells may be complete within one month (40). Evidence of inflammatory reaction in the spinal cord is almost absent after three months (41). Recovery of muscle function has not been so specifically delimited. While clinical tests indicate that improvement may continue for 18 months to two years, most of it occurs in 6 to 10 months (42–44). Electromyographic evidence has shown that recovery in an individual muscle may continue for at least 6 months (45). The point at which metabolic processes in the tissues cease to vary has not been determined. Under these conditions, designation in Figure 1 of the duration of the convalescent phase as 6 months is arbitrary.

In this section, data obtained on 7 patients from the second through the ninth to tenth month serve as the basis for delineation of the changes in metabolism during the convalescent phase. For D.Q., M.O'M., D.A., and J.C., who were studied during the acute phase as well, parts of these data are shown in Figures 2 to 5.

1. *Nitrogen.* Following the peak of nitrogen excretion during the third week, noted in the first four patients, urinary nitrogen gradually decreased for several weeks. In R.W., F.S., and C.R., whose studies were begun between one and two months after onset, gradual decline in nitrogen excretion was also noted. Although studies of the latter three patients were clearly initiated after the peak, the magnitude of their nitrogen loss when first seen is worthy of note. In the fourth week after onset R.W.'s urine contained 19.5 gm. per day of nitrogen and his nitrogen balance was negative to the extent of 7.94 gm. per day; F.S. in the fifth week after onset excreted 19.8 gm. per day in the urine and had a balance of − 6.96 gm. per day.

Nitrogen balance continued to be negative for two to five months despite constant daily protein intakes of 80 to 90 gm. Mean duration of negative nitrogen balance for the 7 patients was 103 days. As might be expected, negative balance persisted longest in those with most severe muscle involvement. Patients began to sit up at approximately the time nitrogen excretion balanced nitrogen intake, and began walking approximately 5 to 6 weeks later. Hence, ambulation did not play a role in return of nitrogen equilibrium. In D.Q., who received only conventional therapy, total nitrogen loss before reestablishment of equilibrium was 308.7 gm., equivalent to a loss of 10 Kg. of muscle protoplasm; actual body weight loss for this patient over the 62 days was 9.5 Kg. Total N losses of M.O'M., D.A., and J.C., partially reduced in some cases by special treatments, were 165.9, 424.6

TABLE II

Summary of data on nitrogen excretion and balance and total serum proteins, during acute and convalescent phases of paralytic acute anterior poliomyelitis

Patient	Maximal urinary nitrogen		Maximal negative balance*	Total nitrogen loss		Time to N equilibrium	Total serum proteins first 4 months	
	Gm./day	Day after onset		First 22 days	To N equilibrium		Mean	S.D.
			gm./day	*gm.*	*gm.*	*no. of days*	*gm. %*	*gm. %*
D. Q.	20.1	16th	−10.7	173.2	308.7	63	6.5	±0.21
M. O'M.	17.5	13th	−10.1	88.7	165.9	88	6.9	±0.19
D. A.	29.8	17th	−17.3	185.2	424.6	93	6.8	±0.44
J. C.	26.4	17th	−10.8	109.5	319.5	76	6.0	±0.45
Mean of 4 Patients	23.5	16th	−12.2	139.2	304.7	80	6.6	±0.41
R. W.					(253.1)†	106	6.4	±0.50
F. S.					(226.9)‡	147	7.2	±0.27
C. R.					(94.8)§	151	7.0	±0.26
Mean of 7 Patients						103	6.7	±0.41

* At time of maximal urinary nitrogen.
† Lacking data on nitrogen loss from onset to 19th day.
‡ Lacking data on nitrogen loss from onset to 30th day.
§ Lacking data on nitrogen loss from onset to 49th day.

and 317.7 gm. Mean total loss for these three patients and D.Q. was 304.7 gm., and the mean duration of their negative N balance was 80 days (Table II).

Return of nitrogen balance to equilibrium was usually followed by a small positive balance lasting for several weeks. This was less than 1 gm. per day except in the least involved patient, D.Q., whose balance at the time he began to walk was + 2.5 gm. per day. As he became more active, his nitrogen balance levelled off at + 0.5 gm. per day. The large early nitrogen loss was never quantitatively restored in any of the patients during the period of study.

Nitrogen to sulphur ratios in the urine after the first month were consistently within the range of 14 : 1 to 16 : 1.

2. *Creatine metabolism.* Fall in urinary *creatinine* excretion continued for from two and one-half to four months, the mean for the group of seven patients being three and one-half months. Extent of the decline was proportional to the degree of muscle involvement. For example, in D.Q. with the least muscle involvement, reduction in urinary creatinine was 31 per cent (in creatinine index, 21 per cent) over a period of two and one-half months. The creatinine of D.A., with the greatest involvement of the four acute cases studied, declined 58 per cent (decline in creatinine index, 51 per cent) over a three and one-half-month period (Table III). Following the phase of gradual decline, urinary creatinine remained virtually level (less than 0.08 gm. increase) except in two patients who showed a greater degree of muscle recovery than the others. In R.W. rise in urinary creatinine was slight, 0.16 gm. or approximately 12 per cent over a 4-month period; in D.Q., 10 months after onset, creatinine excretion had returned to 95 per cent of its initial level.

Day to day variation in urinary creatinine was in most patients less than ± 0.060 gm. In three more severely involved patients, however, values were consistently higher on the days when meat was given in the diet than on the days when the menu was creatine-creatinine-free; when beef was the principal protein source, urinary creatinine in these three patients ranged as much as 0.150 to 0.200 gm. per day higher than the values of days with creatine-creatinine-free menus.

Urinary *creatine* excretion, as has been stated, was extremely high during the third week in three of the four patients whose studies were begun during the acute phase. Patients R.W., F. S., and C.R., whose studies began later, had 24-hour urinary levels of 1.320, 0.980, and 0.880 gm. per day, respectively, when first seen. From these early

TABLE III

Summary of data on creatine and creatinine excretion, changes in body weight and time of fall in leg girth, during acute and convalescent phases of paralytic acute anterior poliomyelitis

Patient	Maximal urinary creatine		Average urinary creatine convalescent phase*	Fall in urinary creatinine			Weight loss			Time of fall in leg girth
								Total loss		
	Gm./day	Day after onset		Gm./day	% of initial	Time in weeks	First 22 days	Amt.	Time in weeks	
			gm./day				*Kg.*	*Kg.*		*weeks*
D. Q.	0.365	17th	0.182	0.45	31	11	6.5	10.3	8	9
M. O'M.	1.080	11th	0.439	0.47	43	13	5.0	7.3	13	13
D. A.	1.780	17th	0.642	1.20	58	14	10.0	12.5	11	10
J. C.	1.605	17th	0.458	0.75	42	18	8.7	15.6	18	17
Mean of 4 Patients	1.208	16th	0.430	0.72	44	14	7.6	11.6	12	12
R. W.			0.524			15			13	11
F. S.			0.759			17			8	21†
C. R.			0.762			17			11	11
Mean of 7 Patients			0.538			15			12	13

* Second through sixth months.
† Time of fall in calf girth 21 weeks, of thigh girth 7 weeks.

high levels urinary creatine gradually declined until a plateau was reached, usually at the time when creatinine excretion stopped decreasing. This new level of urinary creatine ranged from 0.150 to 0.700 gm. per day among the group of seven patients, the mean being approximately 0.440 gm. per day. In several instances there then followed a very gradual shift to a level approximately 0.100 gm. per day above the previous low. Beginning of increased activity, such as sitting and particularly actual ambulation, was attended by a distinct rise in urinary creatine, brief in those patients in whom ambulation could be carried forward progressively, small but sustained in those in whom ambulation had to be very gradual. In a few instances in which there was a rather abrupt beginning of weight-bearing on the legs, the rise in urinary creatine was accompanied by a brief (a few days) but well-marked increase in urinary phosphorus.

In D.Q. and R.W., who in later months were able to walk actively, a gradual fall in urinary creatine occurred. In R.W., however, despite a downward trend to levels as low as 0.223 gm. per day, creatinuria remained variable, ranging between 0.350 and 0.600 gm. per day at discharge nine months after onset. D.Q. had levels of 0.130 to 0.180 gm. per day upon discharge from the hospital six months after onset; upon re-entry for a two weeks' observation after a three and one-half months' interval of active ambulation and exercise, his creatine was at normal levels of 0.120 and 0.049 gm. per day for two 6-day metabolic periods (in which the diet was not creatine-free). D.Q. was the only patient whose urinary creatine returned to normal levels. He was also the only patient whose creatine tolerance returned to normal range; this occurred a little over three months after onset of illness and prior to the beginning of ambulation. In all of the other patients, despite gradually improving muscle function, creatine tolerance tests consistently showed less than 60 per cent retention.

3. *Serum proteins.* Serum total proteins tended to decline slightly and very gradually in six of the seven patients, the fall averaging 0.6 gm. per cent at 4 months and 0.8 gm. per cent by 6 to 7 months. Serum proteins fell below 6.0 gm. per cent in only two patients; in R.W., three of six values during the 7th to 9th months were below 6.0 gm. per cent; in J.C., mean serum protein from the 2nd through the 6th month was 5.8 ± 0.2 gm. per cent S.D., in contrast to the mean of 6.5 gm. per cent for three values during the first six weeks. Withdrawal from all patients of 35 to 40 ml. of blood every two to three weeks for chemistry determinations may have had some influence on the gradual fall in serum proteins.

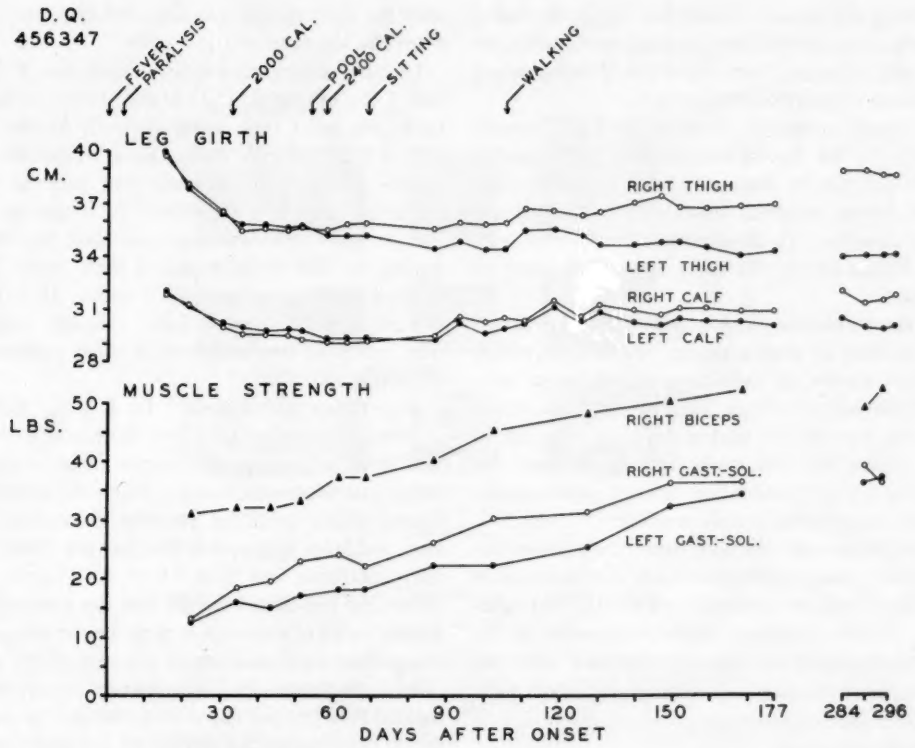

FIG. 7. MEASUREMENTS OF THIGH AND CALF GIRTH AND OF MUSCLE STRENGTH BY ERGOMETER IN THE RIGHT BICEPS AND RIGHT AND LEFT GASTROCNEMIUS-SOLEUS MUSCLES IN PATIENT D.Q. DURING THE FIRST SIX AND TENTH MONTHS AFTER ONSET

In D.A. and J.C., the most severely involved of the patients studied during the acute phase, Howe fractionation of serum proteins during the first two weeks of illness revealed no abnormalities.

4. *Body weight.* Loss in body weight, rapid during the first three weeks, continued at a slower rate for from two to four months after onset. Mean duration of decline in weight for the seven patients was 81 days. For the four whose weights were obtained early in their illness the total weight loss ranged from 7.7 to 15.6 Kg. During this period, except for the first week of illness, dietary intakes were calorically adequate; in later months these same diets maintained weight satisfactorily. With one exception, the period of weight loss in each patient approximately coincided with that of negative nitrogen balance. In R.W., whose loss of weight ceased before nitrogen equilibrium resulted, the diet was raised from 2000 to 2400 calories without change in protein intake three months after onset.

5. *Leg girth.* Measurements of girth of thighs and calves showed a rapid decrease during the first month and a continuing decrease at a slower rate for two to five months, depending upon extent of muscle involvement, the average duration of decrease being twelve weeks. Leg muscle atrophy, indicated by the decline in these measurements, corresponded in time to decrease in body weight. Duration of negative nitrogen balance and of decline in urinary creatinine averaged approximately three weeks longer.

Initial measurements were not taken early enough to permit calculation of the percentile decrease in leg circumference. Indication of its extent may be obtained from a graph of the leg measurements in D.Q. (Figure 7) whose degree of decrease in girth was representative of the group.

Following the period of decrease, leg girth tended to remain constant if involvement was severe, or gradually increased from 2 to 4 cm. if well-marked functional improvement occurred.

6. *Muscle strength.* Periodic testing of muscle strength by the Lovett method was supplemented in five patients by ergometer tests of the strength of the biceps, anterior tibial and gastrocnemius-soleus muscles. In three patients ergometer tests were begun during the third week after onset of illness.

In those muscles which improved most, progressive increase in muscle power was evident within the first month, as early as a second set of tests had been made. Hence, improvement in muscle strength was evident during the time when nitrogen balance was still markedly negative, and decreasing leg girth and falling urinary creatinine indicated progressive muscle wasting.

In all these patients with moderately severe involvement, graphs of improvement in muscle power with time were not parabolic but virtually straight-line. In two patients a slight acceleration in the rate of improvement possibly occurred after the beginning of standing and walking, but minor variations in the improvement of others could not be similarly explained.

Duration of improvement in muscle power was variable. Improvement continued for 5 months in D.Q. (Figure 7), and in D.A. for approximately 7 months. In the other three patients improvement had not slackened when observation was discontinued at 9 to 10 months after onset.

C. *Chronic phase*

The chronic phase of acute anterior poliomyelitis is regarded as that period in which no further significant improvement in muscle function occurs. Metabolic data relative to this phase are afforded mainly by two patients, J.W., observed from the 7th to the 12th month, and R.V.K., observed from the 17th to the 24th month (Figure 8). Most of the data indicate a relatively stable state of metabolism during these later months.

1. *Nitrogen.* In both patients nitrogen balance was slightly positive, averaging approximately + 1 gm. daily. Nitrogen balance in the other patients (in preceding section of paper) observed after the sixth month was also slightly positive but generally less than + 1 gm. daily.

Urinary nitrogen : sulphur ratios for R.V.K. and J.W. averaged 15.1 : 1 and 16.6 : 1, respectively, the latter ratio being distinctly higher than that of other patients during the convalescent and chronic phases. When these two patients were permitted to sit in a wheelchair for longer periods and to stand with crutches and long leg braces during the last three weeks of their study, both showed elevated urinary N : S ratios, 17.3 : 1 for R.V.K. and 18.3 : 1 for J.W. Similar findings were noted on two occasions in other patients on beginning mobilization.

2. *Creatine metabolism.* In R.V.K., urinary *creatinine* averaging 0.20 gm. daily and a creatinine index of 7.3 were indicative of previous extreme loss of muscle tissue. From the creatinine figure, which persisted steadily throughout her stay, and from assumption that her pre-illness urinary creatinine had been 0.8 to 1.0 gm. per day (from her pre-illness weight and the average creatinine index of women), it could be estimated that this patient had lost 75 to 80 per cent of her original muscle mass. In J.W., urinary creatinine remained 0.49 gm. per day during her first ten weeks under observation, but during the last three weeks while she was being mobilized, her creatinine averaged 0.55 gm. per day.

Creatinuria remained consistently 0.500 to 0.600 gm. daily in J.W. and ranged between 0.500 and 0.750 gm. per day in R.V.K. Creatine tolerance tests averaged 35 per cent retention in J.W. and 23 per cent in R.V.K.

Several follow-up observations were made of the urinary creatine and creatinine and creatine tolerance in the four patients who had been studied during the acute phase. A single observation in D.Q., 22 months after onset of illness and a year after he had resumed his studies in medical school, showed a slightly lower urinary creatinine (fall from 1.34 to 1.25 gm. per day), a return of significant creatinuria (0.220 gm. per day) but a continuing normal tolerance (96 per cent). A series of six observations from the 19th to the 43rd month after onset on M.O'M., who during this time was working as a secretary, showed a gradual rise in urinary creatinine from 0.68 to 0.82 gm. per day, a slight fall in creatine (to approximately

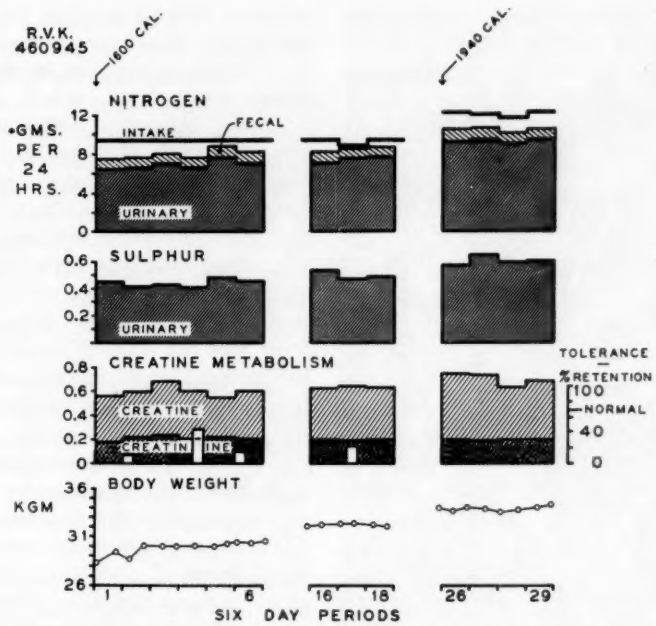

FIG. 8. NITROGEN BALANCE, URINARY SULPHUR, CREATININE AND CREATINE, CREATINE TOLERANCE AND BODY WEIGHT IN PATIENT R.V.K., 19-YEAR-OLD FEMALE, DURING THE 18th, 21st AND 23rd MONTHS AFTER ONSET

As indicated, urinary creatine in this patient exceeded urinary creatinine.

0.200 gm. per day) but no significant change in her impaired creatine tolerance (33 to 67 per cent). A single observation in D.A. 34 months after onset, following continued efforts on the part of the patient to improve his muscle function, showed a slight rise in urinary creatinine from 0.90 to 0.96 gm. per day but no significant change in urinary creatine or in creatine tolerance. J.C., whose muscle involvement was extreme but limited to his legs, returned to work as an art director using a wheelchair; a series of three observations at 10, 18 and 28 months after onset revealed no changes in the three indices.

3. *Body weight.* The body weight of J.W. remained remarkably constant during the five months she was studied. R.V.K., who weighed 28.3 Kg. (62 lbs.) when first admitted, gradually gained weight to 34.4 Kg. (76 lbs.) by the 29th metabolic balance period.

4. *Leg girth.* Thigh and calf circumference of J.W. did not change appreciably during her stay. In R.V.K., who slowly gained weight and continued in positive nitrogen balance, measurements showed a gradual increase of 1.1 cm. in calf circumference and 3.5 cm. in thigh circumference over a 6-month period.

5. *Muscle strength.* Ergometer tests and muscle testing by the Lovett technique revealed no significant change in muscle power in J.W. or R.V.K.

COMMENT

Data from this study make it possible to describe the pattern of a number of changes in metabolism resulting from acute anterior poliomyelitis. This pattern is well outlined for the first nine months after onset of the disease but less completely documented for the period from the 9th to the 24th month.

Degree of nitrogen loss

The most striking feature was the extraordinarily large and prolonged loss of nitrogen. Total nitrogen loss was greater (mean, 305 gm.), lasted longer (mean, 80 days) and the peak of nitrogen

loss occurred later (mean, 16th day) than has been recorded in a number of other conditions known to provoke protein loss, such as other infectious diseases (46–52), fractures and surgical procedures (53–62). In 10 cases of meningitis observed by Grossman, Sappington, Burrows, Lavietes, and Peters (47), total nitrogen loss ranged from 3 to 180 gm. and averaged 65 gm.; negative balance lasted for an average of 9.6 days. In 14 cases of pneumonia studied by Wolf and Lambert (52) the average peak single day urinary nitrogen excretion was approximately the same (23.9 gm.) as in the group of poliomyelitis patients, but this occurred at the sixth day and negative balance was estimated to have lasted for an average of only 10 days. In a group of cases of fracture studied by Howard (58) average total loss of nitrogen was 225 gm., negative nitrogen balance lasted for 35 days and maximum excretion was reached, on an average, 6.6 days after injury.

The large nitrogen loss in paralytic poliomyelitis was ultimately succeeded by a phase of positive balance lasting several weeks. Except in the case of the least involved patient, D.Q., however, this was less than 1 gram daily. The nitrogen loss was never quantitatively restored during the period of study in any of the patients, and, in view of the irreversible character of much of the muscle damage, it is likely that in most of them the nitrogen deficit was permanent.

Origin of nitrogen loss

To account for such an extensive nitrogen deficit in poliomyelitis, several possible mechanisms must be considered. These include diminished caloric or protein intake, fever, "stress," immobilization, and atrophy of muscle tissue resulting from denervation.

When diet is rigidly restricted, gradual utilization of protein stores for energy results in a negative nitrogen balance that may be as great as 10 gm. daily in a previously healthy man (63). This deficit is readily abolished when adequate food is provided, as was shown in normal subjects in an experiment which simulated sudden withdrawal and gradual replacement of food intake generally occurring after surgery or in an infectious disease. Our patients with poliomyelitis had insufficient caloric and protein intakes for only the first week to 10 days after onset so that the influence of inadequate intake *per se* on the nitrogen balance was of brief duration. Food intake had returned to a satisfactory level long before nitrogen loss had become maximal.

"Toxic" destruction of protein in febrile diseases occurs promptly (64) and, if fever is prolonged, may result in losses that are extensive (47) and difficult to overcome with high caloric feeding (48, 65). In patients with brief and uncomplicated fever, nitrogen loss was limited closely to the period of temperature elevation (64). In the four patients whom we studied during the acute phase, fever was not marked (mean high, 38.5°) and had subsided six to nine days after onset, well before nitrogen excretion had approached its maximal level.

A stress or insult unaccompanied by fever, such as a fracture or surgical procedure, is generally followed by excessive excretion of nitrogen (53–62). In such conditions increased urinary and blood levels of 17-hydroxycorticoids have been shown to occur (66, 67). These events have been presumed to be related, with the possibility that nitrogen loss is either caused or permitted by increased secretion of adrenal cortical steroid (67). Paralyzing acute anterior poliomyelitis may involve similar mechanisms of nitrogen loss, but there are regrettably few data currently available which would indicate in poliomyelitis altered secretion of adrenal cortical hormones. Coriell, Siegel, Cook, Murphy, and Stokes (68) have reported low levels of circulating eosinophiles on the third day after onset. In the same series six of eight children had depressed urinary 17-ketosteroid excretion. In our complete group of eleven patients three showed a distinct fall in 17-ketosteroids. These findings contribute little and may merely be in line with previous observations that urinary total 17-ketosteroids in stress are variable, tending to decrease if any change occurs (69). We are not aware of any determination of blood or urinary 17-hydroxycorticoids in patients with poliomyelitis.[3]

[3] Since completion of manuscript, the report of Gemzell (91) has been noted which states that the average daily plasma 17-hydroxycorticoid level in 15 patients with poliomyelitis during the first two weeks did not differ significantly from the levels of normal individuals in the same age range. (Footnote received after manuscript sent to press.)

Although nitrogen excretion in most stress situations (70) simulates the pattern of poliomyelitis seen here, the negative nitrogen balance is of lesser magnitude, the peak loss occurs typically within the first week after trauma, and there is usually a return to nitrogen equilibrium by the third or fourth week. From these considerations it seems probable that processes currently regarded as involved in response to "stress" were operative chiefly only during the first phase of increased nitrogen loss. They fail to provide adequate explanation for the long-continuing negative nitrogen balance.

The study of immobilization on normal healthy young men has made it possible to estimate its relative importance in the excessive nitrogen excretion in poliomyelitis. The normal subjects who were immobilized by means of body plaster casts to a degree approaching that of the patients with paralytic poliomyelitis lost in six to seven weeks a total of 30 to 84 gm. of nitrogen, the mean for the group being 53.7 gm. Although a small peak in nitrogen excretion occurred at 10 days, loss was steady throughout the bed rest period. Hence, immobilization *per se* could be an appreciable factor, but would not account for more than 25 to 30 per cent of the total protein loss in these poliomyelitis patients.

The major source of nitrogen loss, at least after the third week, was presumably protoplasm of muscles whose nerve supply had been affected by the poliomyelitis infection. This inference is supported by the progressive decline in urinary creatinine, by correlation between the degree of decline of this constituent and proportional weight loss and by correlation of actual and theoretical weight losses, the latter calculated from nitrogen balance data.

Decline in urinary creatinine affords a measure of the degree of muscle deterioration and of the length of time during which atrophy occurred. The basis for this statement is that since 98 per cent of the creatine in the body is in muscle, 98 per cent of urinary creatinine may be assumed to reflect the daily conversion of muscle creatine to creatinine; reservation probably should be included that the unknown turnover rate of the remaining 2 per cent might introduce a small error. After initial fluctuations, urinary creatinine excretion rapidly and steadily declined for an average of three and one-half months, then reached a relatively stable plateau for the remainder of the period of observation. The magnitude of the reductions in creatinine excretion and the absolute levels reached were in good correlation with clinical estimates of the extent of muscle involvement based largely on functional criteria.

The magnitude of the reductions in urinary creatinine excretion may also be compared with the loss in body weight from onset of illness until the weight became stable approximately three months later. If it is assumed that on the average the fat and carbohydrate stores of the body in the steady convalescent state ultimately became equal to those in the previously healthy state, actual weight loss would represent decrease in weight of muscle tissue. Percentage decrease in muscle tissue may then be calculated by dividing actual weight loss by the muscle mass at onset. The latter figure can be estimated from the initial body weight using the figure 40 per cent, thought to be the proportion of body weight normally represented by muscle (71). From calculations on this basis, the average decrease in muscle tissue for the four patients studied from onset was 40 per cent (11.6/28.9 Kg.). For comparison, over the same period of time the average reduction in urinary creatinine excretion was 44 per cent.

Additional calculations suggesting muscle atrophy as the major source of nitrogen loss after the third week are afforded by the correlation of actual weight loss with theoretical weight loss determined from nitrogen balance data, based on a number of assumptions suggested by Reifenstein, Albright, and Wells (26). Applying the conversion factor of 1:32 for the weight relation of nitrogen to muscle protoplasm, theoretical weight loss was found to exceed actual weight loss by an average of 13 per cent. Applying the correction for "caloric discrepancy," which assumes that lost nitrogen represents protein made available for energy and hence sparing an isocaloric quantity of fat, the difference was narrowed to approximately 5 per cent.

Another observation which may point to atrophying muscle as the principal source of lost nitrogen was the uniform association, after the third week, of urinary nitrogen and sulphur in the proportion in which these elements occur in muscle tissue, namely, between 14:1 and 16:1. These

values for muscle N : S, however, are based on analyses of muscles in animals and in a very few humans (37, 38), so that this inference must be limited by the reservation that not enough is known of the nitrogen : sulphur ratios in other tissues of the body to conclude that the ratio 14 : 1 — 16 : 1 is distinctive only of muscle.

Elevation of urinary N : S ratio during early phase

Consistent elevation of the urinary N : S ratio during the first two to three weeks of illness suggests that, whatever tissue their source, proteins relatively poor in sulphur were being metabolized. This observation revives old concepts (72, 73) that there may be several types of proteins varying in availability to metabolic processes. These concepts were apparently supported by experiments in which dietary protein levels had been altered and by studies on prolonged fasts (74–76). The convincing demonstration of Schoenheimer and his associates that protein moieties are in a dynamic state has thrown doubt on the existence of separate categories of proteins in the living organism. More recently, however, this same group has obtained data which indicate that the proteins of the plasma, liver and heart turn over more rapidly than do those of muscle (77). From subsequent evaluation of these data Rittenberg (78) suggested that only a small (7 per cent) fraction of muscle protein is metabolically active and that the great mass of muscle is "quite inert, being a mere framework to which are attached certain active proteins."

Determinations of urinary sulphur during infectious diseases and following trauma have been made only sporadically. In the study of typhoid fever by Shaffer and Coleman (48) and in the measurements by Cuthbertson (79) following fractures or orthopedic operations the excretory ratio of N and S was not abnormal. Only in Wolf and Lambert's study (52) of pneumonia in 1910 was a tentative suggestion offered concerning preferential retention of proteins containing higher than usual amounts of sulphur; this impression was gained from the elevation of urinary N : S ratio above 16 : 1 for an average of only three days in six of the fourteen patients. In the present study elevation of the ratio was noted for most of the first two to three weeks. It is of additional interest that high ratios also occurred in a few instances during the late convalescent and chronic phases when standing or sitting was instituted in four severely involved patients.

As we have stated, there is insufficient information in the literature to ascribe the N : S excretion ratio with certainty to muscle fiber composition, and the possibility cannot be excluded that variations in renal handling of large quantities of nitrogen and sulphur may be at least partially responsible. Regardless of the explanation, however, except for the brief suggestion of this phenomenon in the pneumonia study, we are not aware that disproportion between nitrogen and sulphur excretion has been previously documented in infectious diseases or following trauma. It is puzzling that a high N : S ratio should occur during certain stages of paralytic poliomyelitis, a disease specifically involving changes in muscle mass; whereas in infectious diseases and trauma in which a more general tissue breakdown may be present, increased nitrogen excretion is almost exclusively accompanied by the normal nitrogen : sulphur ratios found in muscle tissue.

Origin of initial maximal creatinuria

During the early weeks of the disease, in three of four patients creatinuria reached levels which were considerably higher than previously reported in paralytic poliomyelitis. From consideration of current concepts (80, 81) of the various possible origins of creatinuria, the principal possibilities suggested which might explain the initial peak creatinuria were as follows: increased formation of creatine, alterations in the renal mechanism for excretion of creatine, release of creatine from preformed stores in the affected muscle tissue, and a diminished capacity of the damaged muscle to incorporate the creatine presented to it. Of these various possibilities, the latter mechanism seemed most likely to be principally responsible.

Increased creatine formation during this brief phase seems unlikely from the analyses of urinary glycocyamine excretion which, although slightly greater during the first three weeks than during the convalescent phase, never exceeded normal levels. This failure of the precursor of creatine to exceed normal levels seems particularly significant with regard to creatine formation since from the work of Sims and Seldin (39) one might expect some increase in glycocyamine excretion on the basis of intereference with tubular reabsorption

of glycocyamine by increased tubular loads of creatine.

Renal mechanisms (82) may have had a brief influence during this phase. No information is available bearing on possible variations in glomerular filtration rate during these early weeks. Depressed tubular reabsorption of creatine might occur through competition with similar substances for a common reabsorptive mechanism. Pitts (83) has concluded that excessive amounts of amino acids interfere with tubular reabsorption of creatine from experiments in dogs in which increased creatine excretion occurred as a result of infusions of glycine. Unfortunately we have no data bearing on this point since determinations of urinary amino acid nitrogen were not carried out in our patients. The possibility may be raised that a part of the creatinuria during the peak phase may have been related to alterations in adrenal cortical function, suggested from the fact that creatinuria has been described following trauma of various sorts (79, 84–86) and following the administration of ACTH and cortisone (87). Whether this influence occurs principally through direct muscle or renal mechanisms has not been determined; Zierler, Folk, Magladery, and Lilienthal (82) have shown diminution in tubular reabsorption of creatine on treatment with desoxycorticosterone acetate and in Cushing's disease. The meagre evidences for altered adrenal cortical function in poliomyelitis mentioned previously suggest that such changes occurred earlier in the disease than the period of peak creatinuria in these patients.

Although release or "leakage" of creatine from preformed stores in the affected muscle masses cannot be excluded, quantitative considerations make it seem unlikely that this phenomenon occurred to any great extent. For example, in D.A. who of the early group ultimately lost the greatest proportional amount of muscle mass, approximately 60 per cent, the largest daily excretion of urinary creatine amounted to only 2 per cent of his estimated total creatine mass. This level of creatinuria continued for only six days. During this same period creatine was being provided from formation in amounts which were greater than the amount being excreted. If considerable leakage had occurred, one might expect that at some point during the peak phase creatinuria would have exceeded the initial level of creatinine which, in the normal state, is representative of the amount of creatine being provided to muscle each day. This was not noted in any of the four subjects.

The fourth possibility to account for the high levels of creatinuria is the development of a marked impairment in the ability to incorporate creatine in the considerable mass of damaged muscle. If the body musculature for only a few days were able to retain no more than 10 to 20 per cent of the creatine presented to it from endogenous and dietary sources, this could entirely account for the peak creatinuria in the three patients with the highest levels. [Much less impairment in incorporation is required to explain the lower levels in patient D.Q.] On this basis the subsequent decline in the levels of creatinuria may be explained by improvement in retention of creatine, possibly in conjunction with reduced endogenous formation; the latter process seemed indicated by calculations to be briefly described later. Numerous tests of tolerance to exogenous creatine were performed throughout the studies, the earliest during the fourth and fifth weeks after onset, just following the phase of peak creatinuria. The degree of impaired retention noted in these tests is consistent with the reasoning proposed. Impaired ability of muscle to incorporate creatine most reasonably accounts for the initial high levels of creatinuria. This mechanism is indeed the only one that has been so far demonstrated to occur in muscle disorders accompanied by creatinuria; in progressive muscular dystrophy, isotopic studies by Benedict, Kalinsky, Scarrone, Wertheim, and Stetten (88) have established that this mechanism is responsible for the creatinuria.

For the persistent creatinuria of the *convalescent* and *chronic* phases in the patient with moderate or severe paralysis due to poliomyelitis, presumably a similar explanation holds with the following modification: by the second to third month an actual considerable reduction has developed in muscle mass available for creatine retention, while production persists at normal or reduced though abundant levels.

Rates of creatine formation and conversion to creatinine

The creatine-creatinine data were analyzed in the light of our present understanding of the

processes of creatine formation and conversion. It is regrettable that studies with isotopic creatine were not included; nevertheless, it was possible to utilize the data for calculations which yielded information on the conversion rates of creatine to creatinine and on the constancy of endogenous creatine formation. The data analyzed covered the first four to eleven weeks of the four patients studied during the acute and convalescent phases.

Space does not permit detailed presentation of the calculations but merely an indication of their basis and the results obtained. Effort was made to account quantitatively for the observed day to day decline in urinary creatinine on the basis of a gradual decrease in total body creatine stores. In any individual, total creatine stores (S) are being replenished daily by creatine provided by endogenous formation (C_f) and are being depleted daily through two avenues of loss, conversion to creatinine (C_{con}) and excretion of creatine in the urine (C_{ex}). The latter figure for the calculations was supplied by the analyzed quantities of creatine in the urine. The figure for constant daily creatine formation (C_f) was taken as the pre-illness (or initial) urinary creatinine; this is based on the observations that in health the daily excretion of creatinine is relatively constant and that there is little excretion of creatine in the urine on creatine-creatinine-free diets, leading to the inference that under normal conditions creatine stores are maintained by the endogenous formation of creatine in amounts equivalent to those degraded daily to creatinine. Day by day calculations were made and tabulated of the amounts of creatine daily converted to creatinine and of the changing total body stores of creatine, by the following formulae:

(*1*) $S_{\text{beginning of day}} + C_f - C_{ex} - C_{con} = S_{\text{end of day}}$

(*2*) $C_{con} = S_{\text{beginning of day}} \times ?\ \%$

For each successive day's calculation $S_{\text{beginning of day}}$ was the figure obtained as $S_{\text{end of day}}$ in the preceding day's calculation. Various figures for a constant rate of conversion to creatinine were successively tried; the reasonable range within which to test was provided by the study using isotopic creatine in rats (89) in which 2 per cent of the creatine stores were found to be converted to creatinine daily and by the similar study (90) in one normal man which gave a value of 1.64 per cent. The initial total quantity of muscle creatine, with which the calculations were begun, was obtained by dividing the initial level of urinary creatinine by the percentage conversion being tested. The calculated daily values for creatine loss by conversion (C_{con}) were compared with the declining daily *analyzed* values of urinary creatinine. The correspondence of these two sets of figures provided the test of validity of the various rates of conversion under trial. In certain patients at various stages, changes in creatine formation were invoked to bring about closer alignment of the analyzed and calculated values for creatinine.

The results of these calculations are presented with the realization that their reliability is limited by the assumptions required and by the relative non-specificity of the Folin method, but with the hope that they may stimulate others to investigate these processes further. In one of the four patients, M.O'M., the calculated values for creatinine declined day by day in close agreement with the analyzed values when the calculations were based on a constant conversion rate of 2.2 per cent; the analyzed and calculated data fitted well from onset through the eleventh week when a steady state was reached in which analyzed values for creatine and creatinine and calculated values for creatinine became relatively constant. In the other three patients the analyzed creatinine values agreed moderately well with calculated values during the first two to four weeks at conversion rates varying from 1.2 per cent to 3 per cent. Thereafter the analyzed values fell more rapidly than could be accounted for by any constant conversion rate and constant endogenous creatine formation. Although a progressive reduction in the conversion rate of creatine to creatinine might have occurred, the more reasonable possibility and the one on which agreement was possible for all three patients was the assumption of a continuing constant conversion rate and a decrease in endogenous creatine formation. The analyzed and calculated data of D.A. fitted best with a constant conversion rate of 3 per cent and a reduction in creatine formation of 50 per cent beginning at approximately the 13th day. In J.C., the data fitted best with a constant conversion rate of 1.2 per cent and a reduction of 40 per cent in creatine formation beginning at approximately the 26th day. And in D.Q., the data

fitted best with a constant conversion rate of 3 per cent and a reduction of 30 per cent in creatine formation beginning at approximately the 16th day. At these rates the calculated data in all three patients remained in close agreement with the analyzed data from onset into a steady-state (for both analyzed and calculated data) beginning after the seventh week.

The indication that creatine formation was reduced in these three patients was supported by the fact that from the third month on, the sum of the daily analyzed urinary creatine and creatinine was from 16 to 28 per cent less than the values of the urinary creatinine at onset. In M.O'M, in whose calculations it was not necessary to assume a decrease in creatine formation, the sum of urinary creatine and creatinine in the convalescent phase was approximately equal to the initial values of creatinine, suggesting that in this patient endogenous creatine formation was not reduced.

Muscle power

Results of periodic tests of muscle power with an ergometer emphasized individual variation in duration of improvement. It is generally stated that most of the recovery of muscle function occurs uniformly during the first six to ten months and that with rare exception no further improvement should be anticipated after approximately the sixteenth month. In the present group significant improvement ceased at six to seven months in three patients, while in another three recovery of power continued steadily for more than nine to ten months. Late follow-up determinations of urinary creatine and creatinine in four patients revealed in one a progressive increase in urinary creatinine through the forty-third month. Data in this patient support the observation that significant improvement may occasionally occur as late as three or more years after onset of illness.

Improvement in muscle function was usually unaccompanied by parallel changes in nitrogen or creatine metabolism. During the early weeks it occurred simultaneously with a markedly negative nitrogen balance and decreasing leg girth and urinary creatinine, indicative of progressive muscle wasting. This dissociation is consistent with progressive atrophy of muscle bundles whose neurons were destroyed concurrent with return of function in bundles whose neurons had recovered; subsequent improvement in muscle strength has been related to hypertrophy of surviving muscle bundles. During the later convalescent phase, functional recovery was unaccompanied by a significant gradual rise in urinary creatinine except in two patients. Although we have emphasized the long duration of metabolic changes, particularly in contrast to other disease conditions, relative stability in nitrogen balance and creatinuria was reattained long before cessation of improvement in muscle function.

Time of ambulation

With respect to the proper time to begin ambulation, this report raises a question. Deleterious effects have been described of prolonged immobilization on protein and mineral metabolism and on certain aspects of circulation. On the other hand, the fact that in several of our conservatively managed patients increases in urinary creatine and phosphorus occurred when they began to bear weight suggests the possibility of some danger to muscle tissue from too early ambulation. This question requires further study.

SUMMARY

Metabolic studies were carried out for periods of six to nine months each on nine patients extensively paralyzed by acute anterior poliomyelitis. In four patients observations were begun during the first week of the disease and extended through the convalescent phase. Three patients were studied during the convalescent phase only and two were studied during the chronic phase. The present paper was limited to description of nitrogen and creatine-creatinine data obtained during administration of standard therapy, with the view of delineating the natural history of the disease with respect to these metabolites. Principal findings were as follows:

1. A large and prolonged loss of nitrogen reached its maximum on the average 16 days after onset of illness and lasted two to five months. Mean duration of negative nitrogen balance was 103 days. Average total nitrogen loss (negative balance) of the four patients whose studies were begun within the first week of illness was 304.7 grams (equivalent to 9 Kg. of muscle protoplasm).

2. The major portion of nitrogen loss appeared

to be related to atrophy of muscle tissue, as judged from associated observations of fall in urinary creatinine and decreases in body weight from the third week of illness on. It was estimated that immobilization might be responsible for 25 to 30 per cent of the nitrogen loss. Nitrogen loss of the first three weeks was associated with a high urinary N : S ratio.

3. Creatinuria, developing in the first week of illness, increased rapidly to a maximum during the third week, then declined to persistent intermediate levels averaging 0.440 gm. per day. Of several alternate explanations for the initial peak creatinuria, impaired incorporation of creatine into damaged muscle mass was considered the most likely.

4. Urinary creatinine declined for from two and one-half to four months. The degree of decline ranged from 31 to 58 per cent and was proportional to the extent and severity of muscle involvement.

5. Body weight loss was rapid during the first three weeks and continued for an average of 81 days, despite caloric intakes after the first week which would be adequate under normal circumstances. Weight loss ranged from 7.7 to 15.6 Kg. among the four patients studied from the first week of illness.

6. Muscle strength steadily improved during the phase of negative nitrogen balance and falling urinary creatinine. Results of periodic ergometer tests emphasized variation from one patient to another in the duration of improvement in muscle power. Although improvement in muscle power was generally unaccompanied by parallel changes in nitrogen or creatine excretion, in one patient a gradual increase in urinary creatinine more than three years after onset of disease indicated that significant improvement in muscle function was still occurring at that time.

7. A new, relatively steady state with respect to nitrogen balance and creatinuria was established on the average three to four months after onset. The degree and persistence of the alterations noted in this study evidenced the extent and chronicity of metabolic defects in paralytic poliomyelitis.

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METABOLIC STUDIES IN PARALYTIC ACUTE ANTERIOR POLIOMYELITIS. II. ALTERATIONS IN CALCIUM AND PHOSPHORUS METABOLISM [1]

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Further evidence of the extensive effects of infection with the poliomyelitis virus upon many bodily systems is presented in this paper, the second of a series (1) describing metabolic and physiological features of the natural course of paralytic acute anterior poliomyelitis during its acute, convalescent and chronic phases.

The most marked alteration in metabolism noted during the immobilization of healthy subjects in body casts (2) was greatly increased excretion of calcium, progressing to urinary calcium outputs more than double those of the control phase and to large negative calcium balances. This striking result suggested that it would be pertinent to investigate the extent of mineral loss occurring in patients with a seriously immobilizing disease such as paralytic acute anterior poliomyelitis.

Several indications of alteration in calcium and phosphorus metabolism in poliomyelitis have been reported, such as hypercalcemia, hypercalciuria, urinary tract lithiasis, bone rarefaction and retardation of bone growth. Orr (3) found hypercalcemia four months after onset in a nine-year-old boy with extensive paralysis and, later, in 10 of 11 other children with paralytic poliomyelitis. Wilkins (4) noted "hypercalcemia in some of the paralytic cases at Children's Hospital" in Baltimore. Hypercalciuria in poliomyelitis has been mentioned several times but quantitatively only once, by Wilkins (5) who found as much as 1 gram per day in the urine of a patient, presumably a child, with poliomyelitis. The frequence of urinary tract calculi formation in poliomyelitis is well known, particularly in severely paralyzed patients restricted to respirators (6, 7), although the precise incidence has not been tabulated. Osteoporosis in paralytic poliomyelitis (5, 8–10) has been recognized, but except for our preliminary abstract of these studies (11) accounts have not documented the extent of associated calcium loss. Retardation of bone growth with shortening of the paralyzed limbs of children prior to the age of bone maturity has been shown to be related to the degree of muscle atrophy in the involved extremity and unrelated to the child's age at onset (12, 13).

Interest of recent observations of serum alkaline phosphatase in poliomyelitis has been focused not on bone metabolism but on involvement of enzymatic processes in severe infections. Ainslie, Stumpf, and Francis (14) showed a brief fall in alkaline phosphatase in monkeys with paralytic poliomyelitis or malaria and in cows infected with *Br. abortus*. In 86 humans with poliomyelitis, however, they found no significant lowering during the first two weeks after onset.

The present report outlines the extent and varying pattern of changes in calcium and phosphorus metabolism, principally with respect to the excretion and balance of these elements, during various phases of paralytic poliomyelitis. Particular attention is given to conditions favoring the development of osteoporosis and urinary tract calculi.

PROCEDURE

Plan and procedure of these studies were outlined in the first paper in the series (1). The present report is based on data of the same nine patients, who were studied for from five to nine months on the Metabolism Ward of the Russell Sage Institute of Pathology and New York Hospital. Study was begun early in the acute phase in four of the nine.

Consecutive metabolic data are given in tables in Appendix A. Paralysis ranged in degree and extent from moderately marked to severe. Detailed case summaries are given in Appendix B. Patients' care and therapy

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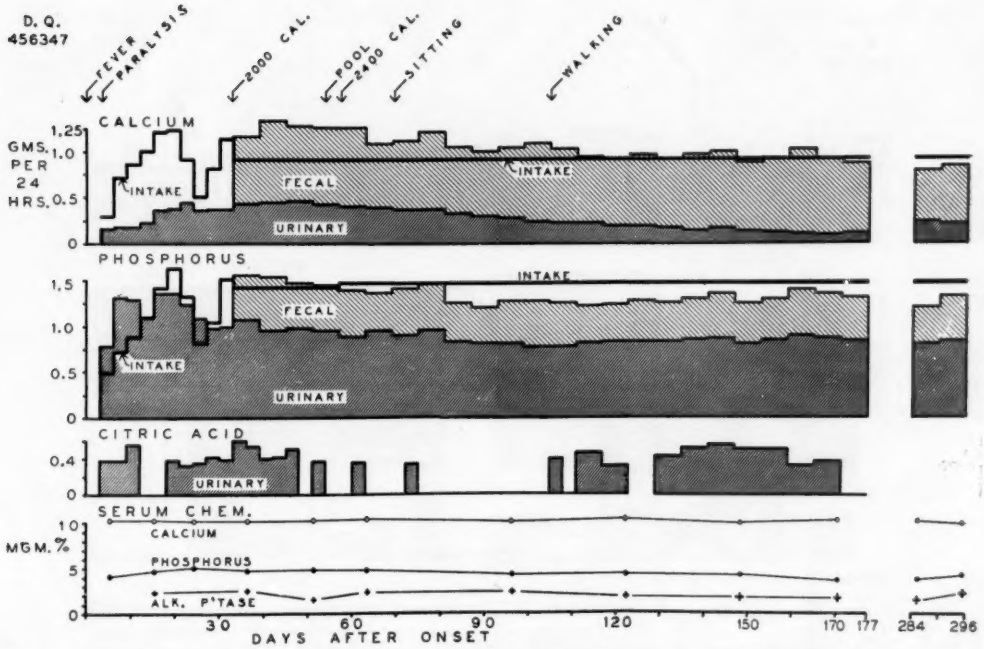

FIG. 1. CALCIUM AND PHOSPHORUS BALANCES, URINARY CITRIC ACID AND SERUM CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE DURING THE FIRST SIX AND TENTH MONTHS OF ILLNESS IN A 21-YEAR-OLD MALE WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS

Serum alkaline phosphatase is plotted in this and in subsequent graphs in Bodansky units. The balance data in this and in subsequent graphs are charted with the intake and output both plotted upward from the base-line and urinary output on the bottom in order to portray clearly changes in urinary excretion as well as changes in balance.

and the constant diets given throughout the studies were described in the first paper of the series (1).

METHODS

Routine of the Metabolism Ward has been described (1, 2). Balance studies were carried out in six-day periods. Initially, urinary calcium and phosphorus were determined daily, but later their analyses and that of citric acid were made on three-day pooled specimens. Methods of analysis of urine, stool and serum for calcium and phosphorus in this laboratory have been previously stated (2). Urinary citric acid was determined by the method of Taussky and Shorr (15, 16). Serum alkaline phosphatase was determined by the method of Bodansky (17, 18) as modified by Woodward (19).

RESULTS

A. *Acute and Convalescent Phases*

Data of acute and convalescent phases (first six months) are considered together since, with the exception of shifts in urinary phosphorus, changes were not rapid. Alterations in calcium in particular were gradual and sluggish, extending over several months. The data of seven patients (Figures 1 to 4) were used as the basis for the following description.

Calcium

a. Pattern and degree of loss. Urinary calcium increased gradually to high levels at the fifth week after onset of illness. Among the seven patients maximal urinary calcium ranged between 0.391 and 0.789 gm. per day (average over a six-day period), the mean maximum for the group being 0.574 gm. per day (Table I). Urinary calcium was maintained at a high plateau, remaining above 0.250 gm. per day for an average of five and one-half months, and declining slowly before ambulation was begun. Fecal calcium excretion was unchanged in four patients and variably increased in three, tending in these latter individuals to be

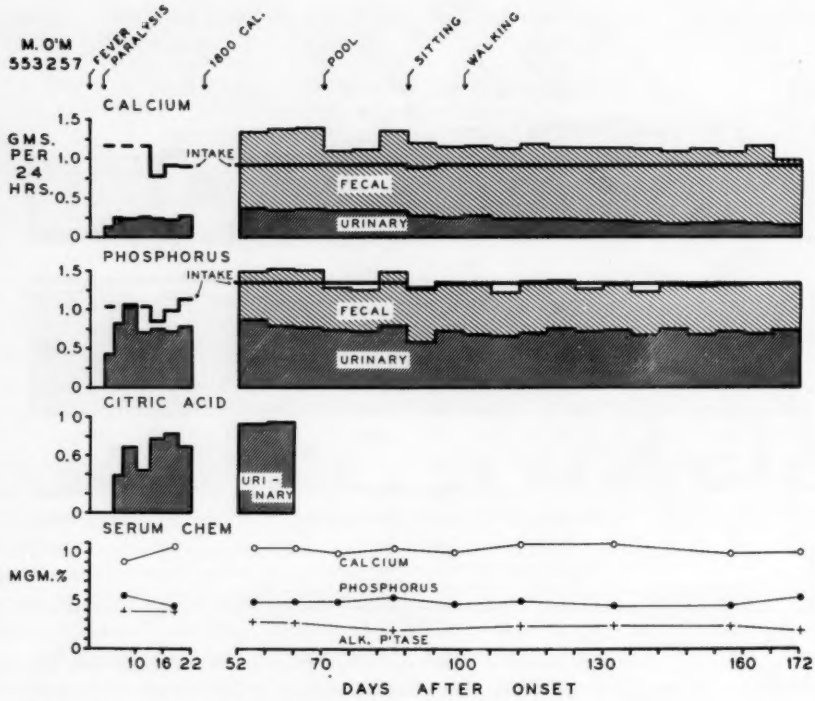

FIG. 2. CALCIUM AND PHOSPHORUS BALANCES, URINARY CITRIC ACID EXCRETION AND SERUM CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE DURING THE FIRST SIX MONTHS OF ILLNESS IN A 21-YEAR-OLD FEMALE WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS

The interruption in this graph and those in subsequent graphs result from the exclusion of data for the periods *during* and for from two to four weeks *following* the administration of special procedures (*see text*).

greatest during the second and third months and remaining within 0.100 gm. per day of the calcium intake figure for approximately six months.

Maximal negative calcium balance occurred on the average nine weeks after onset and ranged from −0.420 to −0.683 gm. per day among the seven patients, the mean being −0.540 gm. per day. Negative calcium balance continued for an average of seven months; positive calcium balance was regained approximately one month after patients began to stand on their feet. Average total calcium loss over the seven months was 58 grams.

b. Relation between total loss and osteoporosis. In view of the widely varying estimates of the percentage loss of total body calcium necessary for osteoporosis to become roentgenographically evident and the lack of specific data on this point, analysis in this respect was made of the total calcium losses of these patients. In the four patients whose studies were begun within one week of onset the entire loss was exactly measured; in R.W. all but the small losses of the first three weeks were known, and in F.S. all but those of the first four weeks.

X-rays of long bones and spine taken at approximately monthly intervals were evaluated by Dr. John Evans, Radiologist-in-Chief of the New York Hospital-Cornell Medical Center, who was unaware of the metabolic data. Initial X-ray evidence of osteoporosis was usually a patchy demineralization and coarsening of trabecular markings in the lower extremities regional to the joints, most often in the necks or distal ends of the femora. In three patients the first abnormality noted was a sub-metaphyseal band of rarefaction in the distal tibiae (Figure 5). These earliest signs of de-

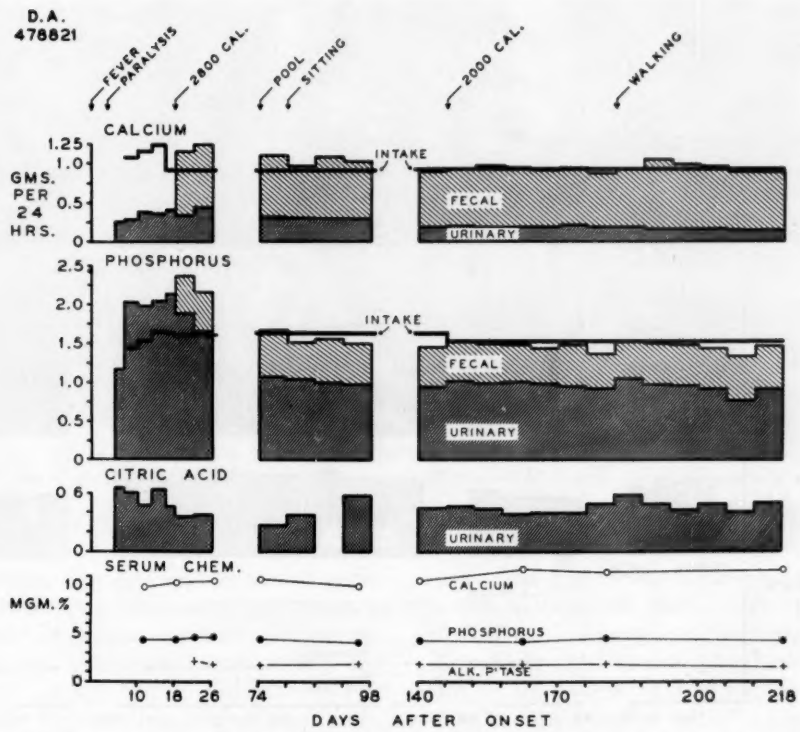

FIG. 3. CALCIUM AND PHOSPHORUS BALANCES, URINARY CITRIC ACID EXCRETION AND SERUM CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE DURING THE FIRST, THIRD, AND FIFTH THROUGH SEVENTH MONTHS OF ILLNESS IN A 27-YEAR-OLD MALE WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS

mineralization appeared approximately three months after onset and occurred with an average loss of 2.0 per cent of the total body calcium. [In Table II in which these data are presented, estimated total body calcium has been taken as 1.8 per cent of pre-illness body weight (20).]

As immobilization continued, areas first noted became more lucent and changes appeared in pubic and ischial rami and in the acetabular fossa of the innominate bone (Figure 6). Although recumbency continued for an average of 6 months, negative calcium balance for 7 months, and total calcium losses averaged 4.6 per cent with range to 9.4 per cent, no changes in the spine could be detected.

Phosphorus

In contrast to the gradual increase in calcium excretion, phosphorus excretion rose promptly, the increase being entirely in the urinary component. Peak excretion occurred between the 9th and 17th days among the four patients studied early, the mean being the 14th day. This was two days prior to the mean day of peak nitrogen excretion and approximately three weeks before maximal calcium output developed.

Maximal urinary phosphorus ranged between 1.07 and 2.13 gm. per day (three-day average) with a mean maximum of 1.64 gm. per day for patients D.Q., M.O'M., D.A., and J.C. Maximal negative balance, at this same time, using in calculations the average fecal phosphorus over the first four periods, ranged from -0.452 to -0.986 gm. per day, the mean being -0.724 gm. per day. Following this early peak, urinary phosphorus excretion declined gradually but remained more than 1.0 gm. per day for an average of three and one-half months. Return to positive phosphorus bal-

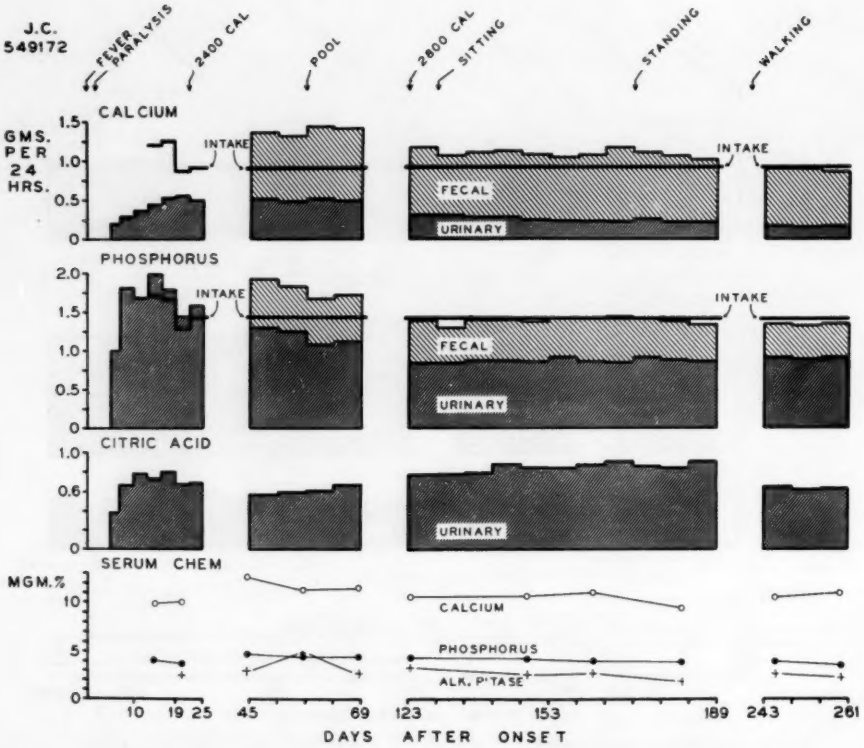

FIG. 4. CALCIUM AND PHOSPHORUS BALANCES, URINARY CITRIC ACID EXCRETION AND SERUM CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE DURING THE FIRST, SECOND, FIFTH, SIXTH AND NINTH MONTHS OF ILLNESS IN A 39-YEAR-OLD MALE WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS

TABLE I

Summary of data on calcium excretion and balance during convalescent phase of paralytic acute anterior poliomyelitis

Patient	Maximal urinary calcium (6-day period)		Mean urinary calcium 2nd–3rd months	Duration urinary Ca over 0.250 gm.	Maximal negative balance		Mean negative balance 2nd–3rd months
	Gm./day	Week after onset			Gm./day	Week after onset	
			gm./day	*months*			*gm./day*
D. O.	0.460	7th	0.401	3.5	−0.420	6th	−0.277
M. O'M.	0.391	5th	0.354	3.5	−0.471	10th	−0.347
D. A.	0.476	5th	(0.307)*	3	−0.429	6th	(−0.124)*
J. C.	0.564	3rd	0.515	5	−0.536	11th	−0.434
	(0.536)	(6th)					
R. W.	0.661	5th	(0.477)*	6	−0.572	6th	(−0.428)*
F. S.	0.674	6th	0.612	8	(−0.663)	(7th)	−0.560
					−0.669	9th	
C. R.	0.789	(9th)‡	0.687	9.5	(−0.665)	(9th)	−0.575
					−0.683	13th	
Mean	0.574	5th	0.514†	5.5	−0.540	9th	−0.439†

* Procedure given during second month which reduced calcium excretion.
† Excluding the data of D. A. and R. W.
‡ Study not started until eighth week.

FIG. 5. INITIAL X-RAY EVIDENCE OF DEVELOPMENT OF OSTEOPOROSIS

On left: X-ray of tibiae of D.Q. taken during first month after onset of illness, showing essentially normal bone density.

On right: X-ray of tibiae of D.Q. taken two months after onset, showing sub-metaphyseal band of rarefaction.

ance, on intakes ranging from 1.35 to 1.66 grams daily, varied widely in time, from eight weeks to nine and one-half months after onset. Phosphorus generally began to be stored approximately two weeks before nitrogen, but in two patients, F.S. and C.R., who had very large calcium losses, phosphorus storage did not occur until two and one-half and five months after nitrogen balance was restored. The total measured phosphorus losses of the first four patients were 18.78, 11.38, 21.12 and 30.97 gm., the mean total loss being 20.56 gm.

Calculation of theoretical phosphorus balances based on the 14.7 : 1 ratio of nitrogen to phosphorus in muscle and on the 2.23 : 1 ratio of calcium to phosphorus in bone (21) revealed that phosphorus was excreted during the acute phase

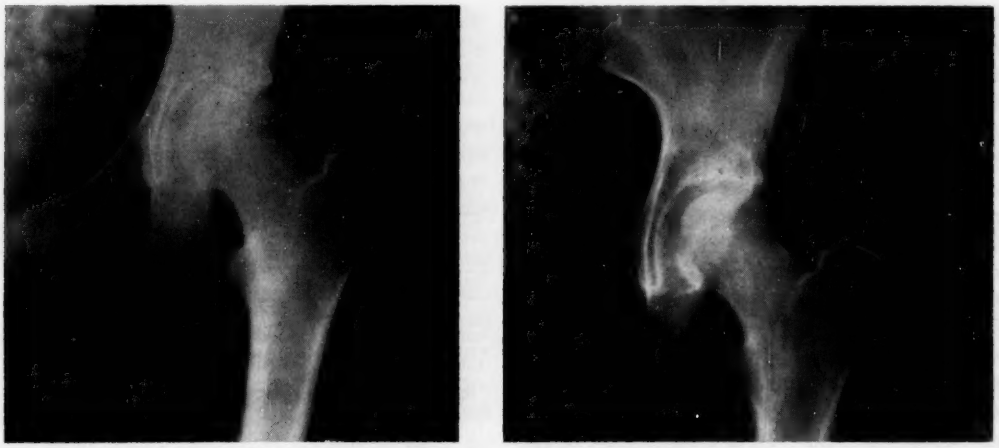

FIG. 6. LATER X-RAY EVIDENCES OF DEVELOPMENT OF OSTEOPOROSIS

On left: X-ray of pelvis and femur of M.O'M. taken during first month after onset of illness, showing essentially normal bone density.

On right: X-ray of pelvis and femur of M.O'M. taken six months after onset, showing rarefaction of pubic ramus, acetabular fossa and neck and intertrochanteric area of femur.

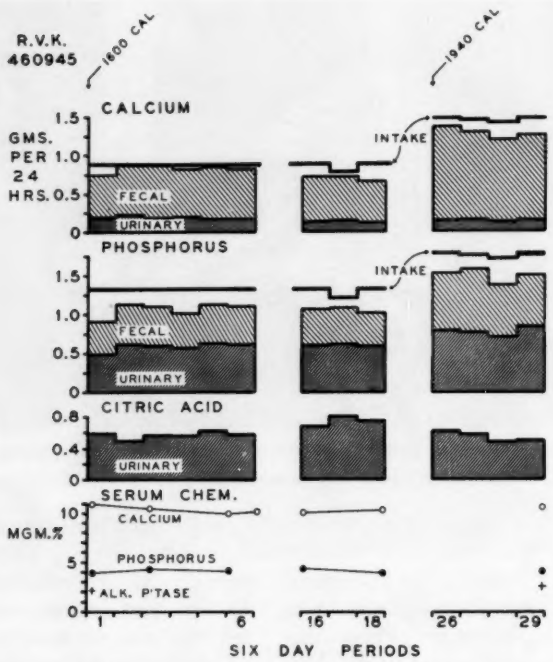

FIG. 7. CALCIUM AND PHOSPHORUS BALANCES, URINARY CITRIC ACID EXCRETION AND SERUM CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE DURING THE 18TH, 21ST AND 23RD MONTHS AFTER ONSET OF ILLNESS IN A 19-YEAR-OLD FEMALE WITH PARALYTIC ACUTE ANTERIOR POLIOMYELITIS

of poliomyelitis in disproportionately greater amounts than nitrogen and calcium. The pattern was consistent from patient to patient. In the four studied early, for a three-day period between the 7th and 10th days after onset actual or measured phosphorus excretion exceeded by 15 to 40 per cent the theoretical excretion based on calcium and nitrogen excretion. A long phase soon followed, averaging 39 days for the first five patients, when theoretical balances were more than 10 per cent more negative than actual phosphorus balances. This phase of retention, or less loss, of phosphorus relative to nitrogen and calcium occurred on the average between the 17th and 56th days after onset and amounted to 15 to 20 per cent, or significantly more than could be accounted for by possible variations in diet. Following this phase, the measured and theoretical phosphorus balances agreed closely, generally within 5 per cent as related to intake. Within this close agreement, measured phosphorus balances from metabolic period to metabolic period were in every instance more positive than the theoretical.

Factors influencing calcium solubility

Among the principal known factors affecting calcium solubility in the urine are urine volume, pH and citric acid. Their role in relation to the problem of urinary tract stone formation has been discussed (*22*).

Citric acid is valuable because it forms with calcium a weakly ionized and very soluble calcium citrate complex. Formation of this complex evidently exerts a protective action in ambulatory patients in whom variations in calcium excretion induced by changes in diet are paralleled by proportional variations in citric acid excretion (*22*). However, when urinary calcium and phosphorus excretion increased during immobilization of normal subjects (*2*), urinary citric acid did not change

TABLE II

Quantitative relationship between calcium loss and demonstrable osteoporosis in paralytic acute anterior poliomyelitis

Patient	Pre-illness body weight	Est. total body Ca*	Initial long bone osteoporosis			Total months recumbent	Total calcium loss		
			Months after onset	Ca loss	Ca loss		Duration	Amount	%
	Kg.	*gm.*		*gm.*	%		*mos.*	*gm.*	
D. Q.	61	1,098	2	18.0	1.6	3.5	5	27.0	2.5
M. O'M.	57	1,026	3	27.1	2.6	3.5	5.5	43.0	4.2
D. A.	89	1,602	3.5	15.4	1.0	6	7	17.0	1.1
J. C.	82	1,476	2.5	27.6	1.9	7	7	54.9	3.7
R. W.	68	1,224	3.5	36.0	2.9	5	8	55.3	4.5
F. S.	82	1,476	3	33.1	2.2	8	8.5	95.5	6.5
Mean of 6 Patients			3	26.2	2.0	5.5	7	48.8	3.8
C. R.	67	1,206	2	(18.0)†	(1.5)†	9	10	113.4	9.4
Mean of 7 Patients						6	7	58.0	4.6

* See text and reference 20 for estimation as per cent of body weight.

† Including an estimate of calcium loss of first six weeks of illness based on rate of loss thereafter and taking into account the usual smaller losses during the first three weeks.

and urinary pH shifted slightly toward alkalinity, thus presenting conditions tending to favor precipitation of calcium phosphate. Urinary calcium in these subjects more than doubled without any compensatory rise in urinary citric acid.

In the patients immobilized with paralytic poliomyelitis a similar lack of compensatory rise in citric acid was evident, except in M.O'M., the only female who was studied during the early weeks. Her average urinary citric acid excretion during the first two weeks of illness was 0.505 gm. per day; during the fifth to seventh weeks when urinary calcium was highest (0.329 to 0.391 gm. per day) her citric acid nearly doubled and during the second and third months averaged 0.954 gm. per day (S.D. = ± 0.048), a urinary citric acid/calcium ratio of 2.7 : 1. In the three males who were studied early, citric acid did not increase during the rise in urinary calcium excretion. For the six males studied during the convalescent phase, during the second and third months when mean urinary calcium was highest, 0.407 to 0.672 gm. per day, the urinary citric acid/calcium ratios ranged from 0.75 : 1 for R.W. to 1.31 : 1 for F.S., the mean ratio for the group being 1.11 : 1.

During acute and convalescent phases urinary pH remained consistently within the normal range and no trends in urinary volume were apparent.

Blood chemistry

The alterations in serum calcium and phosphorus during the first four months after onset are shown in Table III. With one exception, the *mean* serum calcium values were within normal limits for our laboratory, 9 to 11.5 mgm. per cent. In C.R., a 17-year-old boy, the mean was 11.9 with individual values ranging to 14.0 mgm. per cent. In J.C., a 39-year-old man, three of the nine serum calciums obtained during this phase were above 11.5 mgm. per cent. During the phase of hypercalcemia in these two patients urinary calcium averaged 0.687 and 0.490 gm. per day, respectively. After the first four months, with rare exception, serum calcium remained within normal limits.

In each patient a considerable number of values for serum phosphorus were higher than 4.5 mgm. per cent, the mean during the first four months exceeding this figure in four of the seven patients.

Serum alkaline phosphatase generally remained within a normal range of 2 to 4.5 units. Values tended to be low in D.Q and D.A. whose calcium losses were less than the other patients. In C.R., whose calcium loss was greatest, ten values during the fifth to ninth months (not shown in Table III) ranged from 4.2 to 7.1, mean 5.5 units.

In view of the reported tendency for hypercalcemia to accentuate renal injury (4), it may be

TABLE III

Summary of serum chemistry data during first four months after onset of poliomyelitis

Patient	Serum calcium			Serum phosphorus			Serum alk. phosphatase			
									No. of det.	
	Mean	S.D.	No. of det. over 11.5/ total no.	Mean	S.D.	No. of det. over 5.0/ total no.	Mean	S.D.	Under 2.0/ total no.	Over 4.5/ total no
	mgm. %	*mgm. %*		*mgm. %*	*mgm. %*		*mgm. %*	*mgm. %*		
D. Q.	10.2	±0.11	0/8	4.4	±0.26	0/8	2.0	±0.42	2/6	0/6
M. O'M.	10.3	±0.52	0/10	5.0	±0.37	4/10	3.1	±0.66	0/5	0/5
D. A.	10.4	±0.40	0/12	4.4	±0.30	0/12	1.8	±0.53	7/9	0/9
J. C.	11.0	±0.83	3/9	4.2	±0.32	0/9	2.9	±0.88	0/7	1/7
R. W.	10.9	±0.51	1/8	4.9	±0.43	3/8	3.9	±0.43	0/7	1/7
F. S.	11.2	±0.95	1/10	4.6	±0.30	1/10	4.1	±0.90	0/6	3/6
C. R.	11.9	±0.94	4/7	5.1	±0.21	6/7	3.6	±0.63	0/5	1/5
Mean	10.8	±0.60		4.7	±0.35		3.1	±0.90		

noteworthy that blood urea nitrogen determinations performed bi-weekly were never elevated and no defect in urine concentration became apparent.

B. *Chronic Phase*

Two patients, J.W. and R.V.K. (Figure 7), were studied in the chronic phase (more than six months after onset of illness).

Calcium

When J.W. was first observed seven months after onset, urinary calcium was elevated, 0.301 gm. per day, and calcium balance was negative. By the following month urinary calcium had levelled to approximately 0.200 gm. per day and calcium balance was no longer negative; ambulation was unrelated as it was not begun until three and one-half months later. The time at which these indices returned to normal was within the range of the seven patients previously described. Thereafter, urinary calcium remained normal and calcium balance in equilibrium.

In R.V.K., studied between the 17th and 24th months after onset, urinary calcium remained well within normal limits and calcium balance was slightly positive. Her activity was limited to two hours daily in a wheel chair until the end of her studies.

Extensive and marked osteoporosis of pelvis and lower extremities in both patients at the time they were first observed indicated earlier extensive mineral loss which had gradually subsided, long prior to ambulation.

Phosphorus

Throughout these later months, phosphorus balance remained positive to the extent of 0.100 to 0.400 gm. daily. Urinary phosphorus was within normal limits. Theoretical phosphorus balances calculated from balances of nitrogen and calcium deviated less than 10 per cent from measured balances.

Factors influencing calcium solubility

Urinary citric acid excretion did not change during the chronic phase. Urinary citric acid/calcium ratios were a high 4.0 and 3.6, respectively, in these two female patients. Urine volumes and urinary pH were not altered from the normal.

Blood chemistry

Serum calcium, phosphorus and alkaline phosphatase determinations in both patients were consistently normal.

COMMENT

Calcium loss in these paralytic patients was greater than has been previously documented for any other condition, including immobilization for fracture (*vide infra*). Immobilization or disuse appeared to be the major factor in the demineralization, although stress involving adrenal cortical hormone hypersecretion and the trophic influence of denervation may have been contributory.

Influence of stress

In our patients stress mediated through the adrenal cortex probably exerted only a minor in-

fluence. Meagre evidence to date suggests that in poliomyelitis adrenal cortical hyperfunction continues for only a short period (1), while demineralization definitely continued for many months. Furthermore, from evidence in the literature, it is difficult to evaluate the effect of adrenal cortical stress on calcium excretion in man, particularly since in clinical states stress is often accompanied by immobilization. Courses of administration of ACTH (usually 100 mgm. daily) in ambulatory patients in whom calcium excretion has been studied (23, 24) have been brief, 5 to 12 days. Slight increases in urinary calcium were reported in five patients and virtually no change in twelve; fecal calcium excretion was apparently increased in five patients, but in each ACTH was given for 5 days or less. Augmented urinary calcium excretion was noted in only one of three patients receiving 100 to 200 mgm. of cortisone daily for as long as thirty days (25). That significant mineral losses may occur when cortisone is given over longer periods is strongly suggested by an increase in severity of osteoporosis of the spine in a patient with rheumatoid arthritis (26) and by persistent negative calcium balance in a similar patient (27). Only the short-term studies seem applicable to the present consideration, however, in view of probable limitation of stress in poliomyelitis to the first two weeks after onset; evidence that stress may exert an effect for a longer period in this disease has not yet been demonstrated. Studies to date have not consistently shown increased calcium excretion under short-term influence of adrenal cortical hypersecretion.

Influence of denervation

Denervation is usually accompanied by a considerable degree of disuse, but the latter may be minimized by passive motion or weight-bearing. A circumstance in our study in which effects of disuse apparently could be separated from those of denervation occurred in four patients who had severe paralysis of upper extremities. Only one (F.S.) showed demineralization of arm bones and that only to a mild extent; on the other hand, demineralization was uniformly present in femora and tibiae even though lower extremity paralysis was in several instances no more marked than upper. It is suggested that upper extremities, though severely paralyzed, rarely became demineralized because they were sufficiently used or moved.

That denervation *per se* probably does not cause extensive calcium loss is further supported by the observation by Abramson (28) on the effect of weight-bearing in two groups of paraplegics. Over a three-year period one group moved about only by wheel chair while the other walked with braces and crutches for an average of one hour daily. Twenty-five of the thirty non-ambulatory paraplegics showed osteoporosis of the pelvis and legs, whereas only one of the eight who walked with similar injury to the spinal cord showed bone rarefaction and that to only a mild degree.

Although these observations indicate that denervation could account for little, if any, of the extensive calcium loss in our patients, they are too limited to be conclusive. Recent study of urinary calcium excretion in poliomyelitis by Plum and Dunning (29, 30) showed hypercalciuria during early weeks in patients with bulbar or high spinal involvement but without extensive trunk or lower extremity paralysis, and these investigators have discussed (30) the possible role of neural factors in calcium loss. In their studies, an initial pattern was noted of gradual rise of urinary calcium over a period of several weeks, similar to that seen in the present study, indicating that movements of calcium are sluggish, apparently regardless of cause.

Influence of immobilization

Changes noted in this study of poliomyelitis are strikingly similar to those observed in other diseases in which immobilization has played an important role. The changes may be compared with those noted on immobilization of a 14-year-old boy following fracture of a femur treated with open reduction and plate (3), of a thirty-five-year-old women after fusion of an ankle (31), of two cases of Paget's following fracture (32), and of thirteen patients with prolonged dysfunction of a leg, nine of whom were tuberculous (33). The detailed study by Howard, Parson, and Bigham (5) of seventeen male patients immobilized with complete leg and partial body casts for fracture or osteotomy was extensive enough to outline a pattern of calcium loss. In this study, urinary calcium gradually rose during the first month to a

high plateau level, averaging 0.510 gm. per day, which was maintained for sixty to one-hundred days; urinary calcium began to fall shortly before ambulation was instituted.

More direct evidence of the effects of immobilization *per se* was afforded by the study by Deitrick, Whedon, and Shorr (2) of normal healthy men with no previous bone disease. For six to seven weeks four subjects were immobilized (in plaster) to a degree approaching that of a patient such as D.Q. with extensive paralysis of the legs. While the subjects' dietary intake was kept constant, urinary calcium excretion increased gradually until at the fourth to fifth week it reached a peak, averaging 0.342 gm. per day, which was maintained for the remainder of the immobilization. Negative calcium balances were also of considerable magnitude. Maximal urinary calcium excretion in the fracture series was approximately 50 per cent greater and in the poliomyelitis patients was 67 per cent greater than in the immobilized normal subjects. The pattern of loss, however, was almost identical in the three groups, in which immobilization was an outstanding feature.

In the present group of patients, all of whom were extensively paralyzed, magnitude and particularly duration of calcium loss were in proportion to the degree of paralysis and to the degree and duration of immobilization, as is evident from inspection of the two summarizing tables (Tables I and II). In partial contrast to this observation, the study of urinary calcium excretion by Plum and Dunning (29, 30) of twenty-three patients with a wide variety of paralytic involvement due to poliomyelitis revealed little relationship between *intensity* of hypercalciuria and the site and degree of paralysis. *Duration* of hypercalciuria, however, was proportional to the degree of paralysis and to the amount of resulting immobilization.

Mode of action of immobilization

In the absence of weight-bearing, the skeleton is not exposed to stresses and strains that are thought to stimulate formation of bone in the interlacing arch-like patterns of cancellous trabeculae in long bones. Both absence of weight-bearing and muscle paralysis tend to reduce muscle-tendon pull on periosteal surfaces and so diminish the stimuli accentuating bone formation over bone resorption. Abramson's study (28) of paraplegics seems to indicate that absence of skeletal weight-bearing is the more important factor since weight-bearing prevented osteoporosis of pelvis and lower extremities of completely paralyzed ambulatory patients.

The fundamental mechanism by which immobilization permits calcium loss is presumed to be decreased bone formation, with bone resorption continuing at a normal rate, rather than an increased bone resorption such as might be caused by hypersecretion of parathyroid hormone. The tendency to elevation of serum phosphorus during the course of the demineralization seems to exclude the possibility that the parathyroid glands were exerting an abnormal influence.

Possible effects of circulatory changes

Effects on mineral loss of possible changes in circulation to extremities and to bone during immobilization or following neurological injury are difficult to assess. Hyperemic circulatory changes have been reported to improve bone growth, as following sympathectomy (34) and in long standing arteriovenous fistulae (35), and to accelerate fracture healing as in experimental venous ligation (36). A conflicting view (37) has been expressed, however, that decreased blood supply results in increased density of bone and hyperemia leads to skeletal demineralization. It is not known whether changes in bone circulation occur during the paralytic stages of poliomyelitis, and, if so, what kind.

Relationship of calcium loss to development of demonstrable osteoporosis

The principal consequence of the large mineral loss was the development of bone rarefaction or osteoporosis. The important features were localization of its initial evidences in the ends of the long bones of the lower extremities, its appearance as early as three months after onset and the rather small calcium loss in relation to total body calcium, 2.0 per cent. It has been suggested that if non-paralyzed areas were excluded from the estimation of total body calcium used in this calculation, on the assumption that calcium loss and osteoporosis would develop only in paralyzed areas, this would increase the percentage figure

of calcium loss before osteoporosis could be diagnosed by X-ray. Although this is true, the average figure would be raised relatively little since in this group of patients only the skull, ribs and in two instances the upper extremities could be considered unparalyzed.

The sharp band of rarefaction in the sub-metaphyseal area of the distal tibiae of three patients indicated extremely rapid demineralization in a location where bone turnover is normally particularly active. As mineral losses continued, rarefaction became apparent in the pelvis and, in one instance, in the humerus of an extremely paralyzed upper extremity. No evidence of demineralization in the spine appeared although total calcium losses ranged as high as 9.4 per cent. That early stages of demineralization were present in this area is suggested by observation of other patients with poliomyelitic paralysis in whom demineralization of the spine was detected after approximately one year. Since these patients were not on metabolic study, no accurate estimate can be made of the percentage loss of calcium necessary for obvious changes in this area. By comparison with losses of the metabolic group, however, it may be supposed that demonstration of osteoporosis of the spine requires calcium losses of more than 10 per cent. The figure may actually be much higher because of the difficulty of radiological interpretation of the spine due to the fine architecture of the vertebrae and interference by varied pattern of overlying bowel shadows.

Urinary tract calculi formation as a potentially serious consequence of immobilization

The high incidence of urinary tract calculi during immobilizing conditions is well known (5, 38–43) and is documented in Freeman's series (44) of 700 patients with spinal cord injury as 23 to 35 per cent. Certain factors which favor the precipitation of calcium phosphate in the urinary tract were shown to be present during the immobilization of normal men (2). These were excessive excretion of phosphorus as well as calcium, slight alkaline shift of pH and a failure of urinary citric acid to rise compensatorily with calcium as would be expected in ambulatory individuals. In the present study similar factors were evident in that urinary calcium and phosphorus were considerably increased and in the six male patients urinary citric acid failed to rise. There were no significant changes in urinary pH or volume. In the single female patient, increase in urinary calcium was accompanied by marked increase in urinary citric acid, a change which would aid in keeping calcium and phosphorus soluble. The increase in urinary citric acid in this patient is unexplained; it apparently was not related to the increase normally occurring in women during the menstrual cycle, presumably under the influence of estrogenic hormone (45, 46), since this patient had no vaginal bleeding during her hospitalization and no evidence of ovarian cyclic activity in vaginal smears.

In the patients here reported, no calculus developed, and the search for crystals and red blood cells in the urine was negative. Possibly calculi did not form because no other factors conducive to their formation were present. Urinary volumes were not permitted to fall to low levels. Except for brief intervals in two patients, infection, which would have reduced citric acid levels, did not occur; necessity for bacteria or a foreign body as a nucleus for precipitation of salts in the urinary tract has been emphasized (47) in the pathogenesis of nephrolithiasis. Obstruction or stasis (6, 7) presumably by kinking of a ureter, which has also been noted as factor, may have been prevented by frequent changes in position and movement brought about by physical therapy.

Another potential consequence to large mineral losses is renal injury occurring in conjunction with hypercalcemia (4), with attendant damage to renal tubules, albuminuria and reduced ability to concentrate urine. Evidence of impaired renal function was not detected in the present study.

Serum calcium and alkaline phosphatase

Hypercalcemia (serum calcium > 11.5 mgm. per cent) was noted at some time during the course of these observations in four of the seven patients. In two of the four, however, elevation occurred in only a single determination. No relation was found between age of the patient and occurrence of hypercalcemia. Correlation was highly significant, however, for the whole group between mean serum calcium values and total calcium loss (corr. coeff. $= + 0.809$), maximum

negative calcium balance (+ 0.809) and maximal urinary calcium over a six-day period (+ 0.823).

Although serum alkaline phosphatase values generally remained within normal limits, they tended to be low in the two patients with the smallest total calcium losses, and some elevated values were noted in the two patients with the greatest losses. Ambulation was not a factor, since it was not begun until after these alkaline phosphatase changes had developed.

Possible significance of changes in phosphorus excretion

Changes in phosphorus excretion in relation to calcium and nitrogen were of unusual interest because of the high concentration of phosphorus in nervous tissue (48). Calculations of expected phosphorus excretion on the basis of the ratios of this element to N in muscle and Ca in bone indicated a disproportionate actual loss of phosphorus by 15 to 40 per cent between the seventh and tenth days and a relative retention over the succeeding six weeks. These changes paralleled closely with slight delay pathological events in the neurons of the spinal cord and brain (49, 50). Maximum destruction of motor nerve cells is complete within the first 3 to 6 days; thereafter, the proportion of normal motor neurons increases and of abnormal motor neurons decreases gradually for between four and six weeks, indicating recovery of abnormal cells. It seems not unlikely from consideration of quantitative relationships that early excess phosphorus excretion could have come mainly from destroyed or damaged nervous tissue and that later retention represented storage in recovering motor neurons. Abundance of phosphorus in other tissues, however, makes it difficult to exclude the possibility of influence of other factors in the movement of phosphorus.

Influence of ambulation

In view of the apparent relationship of immobilization to mineral loss in this and in preceding studies, the data were surveyed for evidence of an effect on calcium in the opposite direction by ambulation. Decreases in the excretion of calcium occurred during the later convalescent months, but they could be at most only partially ascribed to increased activity and ambulation, since these decreases began uniformly prior to standing and walking; this apparently spontaneous decline in mineral loss suggested the possibility that there were stores of readily available calcium which were beginning to be depleted and that the remaining calcium in the skeleton was more tightly bound. With the beginning of ambulation, calcium excretion continued to decline gradually without evidence of more rapid fall. In other studies some influence of exertion on calcium excretion has been noted. Heavy resistance exercises reduced calcium loss in one of two patients with rheumatoid arthritis (51). A definite reductive influence of ambulation on excessive mineral loss was seen in the recovery phase of the immobilization study of normal men (2) in which ambulation was rapid, but in this study also return of calcium excretion to control levels was sluggish. It can only be concluded in the present study that a moderating effect on calcium of increased activity and ambulation, though possible, was not visible.

The question may be raised that if ambulation had been started earlier and allowed to proceed rapidly, it might have had an evident effect on calcium excretion. Ambulation was generally delayed because muscle recovery had not yet progressed to the point at which it could be expected to provide effective support of the skeleton and effective motility. Actually, some of the data suggested the possibility that too early ambulation might be harmful; brief but definite increases in urinary phosphorus and creatine were noted in the few instances in which ambulation was attempted abruptly. Others have observed *increases* in urinary calcium on early weight-bearing (52), although such changes were not evident in the present study. Finally, careful clinicians warn against too early ambulation (53, 54); premature weight-bearing apparently does not hasten the return of muscle power but may retard it and lead to harmful muscle substitution. When the patient has extensive paralysis, to minimize demineralization measures other than early ambulation evidently must be sought.

SUMMARY

This second paper of a series describing alterations in metabolism in paralytic acute anterior

poliomyelitis outlined changes in calcium and phosphorus. Data were based on studies over six to nine months each of nine extensively paralyzed patients. Four were studied during the acute and convalescent phases of the disease, three during the convalescent phase only and two during the chronic phase. Calcium and phosphorus data presented in this paper were limited to those obtained during the administration of standard therapy, with the view of outlining the natural course of the disease with respect to these elements. Principal findings were as follows:

1. Progressive increase in calcium excretion was noted, primarily in the urinary component. Urinary calcium reached high levels at the fifth week after onset of illness which were sustained in plateau fashion for many months. Maximal urinary calcium excretion averaged 0.572 gm. per day; mean maximal negative calcium balance was − 0.540 gm. per day (constant calcium intake 0.920 gm. per day). Calcium balance remained negative for an average of seven months, mean total calcium loss being 58 gm.

2. The extensive calcium loss seemed to be related principally to the extreme degree of immobilization, an important aspect of which presumably was the lack of skeletal weight-bearing. Possible additional influences on calcium loss of denervation *per se,* of altered circulation to bone, and of stress mediated through the adrenal cortex were discussed.

3. The quantitative relationship was explored between total calcium loss and development of demonstrable osteoporosis, which initially appeared on the average three months after onset near joints of the long bones in the lower extremities. Average total calcium loss was 2.0 per cent of the estimated total body calcium at the time osteoporosis was initially detectable.

4. Increases in urinary excretion of calcium and phosphorus accompanied by little change in urinary volume, pH and citric acid excretion provided a suitable setting for urinary tract calculus formation in the male patients. In the single female patient studied during acute and convalescent phases, urinary citric acid excretion increased markedly, an event which would favor solubility of the increased amounts of mineral in the urine.

5. Serum calcium tended to rise in four of the seven patients studied during acute and convalescent phases; levels of serum calcium were directly related to degree of calcium loss.

6. Phosphorus excretion increased rapidly after onset reaching a peak on the 14th day, approximately three weeks prior to the time of maximal output of calcium. Calculation of theoretical phosphorus balances based on nitrogen and calcium balances indicated a disproportionate actual phosphorus loss during the second week and relative retention over the succeeding six weeks; the time parallel between these alterations and the pathological changes in phosphorus-rich nerve tissue was noted.

7. Observations during the chronic phase of paralytic poliomyelitis revealed essentially normal findings with respect to calcium and phosphorus excretion and blood levels.

8. Ambulation was not observed to exert a definite moderating influence on mineral loss. Modest evidence supported the suggestion of hazard in too early ambulation.

ACKNOWLEDGMENT

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METABOLIC STUDIES IN PARALYTIC ACUTE ANTERIOR POLIOMYELITIS. III. METABOLIC AND CIRCULATORY EFFECTS OF THE SLOWLY OSCILLATING BED[1]

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WITH THE TECHNICAL ASSISTANCE
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As the result of studies with normal subjects (1), the Sanders (2) oscillating bed was suggested in 1949 as a possible aid in preventing or minimizing the deteriorating effects of long immobilization. These studies showed that, during confinement to bed in casts for five weeks, healthy young men lost approximately one-half as much calcium and had less impairment in various physiological functions if placed on oscillating beds than if allowed to remain relatively motionless on ordinary hospital beds. It was pointed out that the usefulness of the Sanders bed in the management of immobilizing clinical conditions could only be determined by careful trial. The present paper reports the results of such a trial in severely paralytic acute anterior poliomyelitis.

The oscillating bed has a motorized gear device which gives it a *slowly* rocking head-to-foot motion. One and one-half minutes are required for the bed to tilt at a barely perceptible speed from the horizontal through 20 degrees to a head up-feet down position and to return to the original horizontal position. This bed should not be confused with the "rapid rocking bed" which rocks at rates of 16 to 30 per minute and is used in weaning poliomyelitis patients with respiratory involvement from tank respirators; nor should this bed be confused with vibrating mattresses or other devices which bring no movement to a bed. The oscillating bed's slow movement has been used for several years to increase peripheral circulation in patients with occlusive arterial disease of the extremities (3–6) and to some extent in patients with phlebothrombosis and thrombophlebitis (7, 8).

A study of the possible value of the slowly oscillating bed in another immobilizing clinical condition has already been made. Wyse and Pattee (9) observed the effects of oscillation in four patients with paraplegia one, two, four and thirteen months after the onset of their disease; the oscillating bed was used for eight hours a day for 12 days in three patients and for 20 days in the fourth. No change in urinary calcium was noted in three of the four patients. A gradual fall in urinary calcium (from 0.515 to 0.307 gm. per day) was noted over a twelve-day period in the patient studied two months after the onset of a transverse myelitis at D-6 and 7, but this fall was not considered significant since urinary calcium continued to decline after oscillation was stopped and remained at a low level (0.150 to 0.200 gm. per day) thereafter. Difficulty in collecting stools in their patients impaired interpretation of fecal calcium data and calcium balances. The oscillating bed was of value in restoring vasomotor tone of paralyzed extremities so that tendency to syncope was lessened when patients were placed in an upright position on a tilt table.

PROCEDURE

The general plan and procedure of the poliomyelitis studies were outlined in the first paper in this series (10). The present report is based on the data of eight patients. Six (M.O'M., J.C., F.S., C.R., J.W., and R.V.K.) were investigated on the Metabolism Ward where closely controlled balance studies were carried out.

The other two patients (R.Wil. and J.L.) were studied for seven and eight weeks, respectively, on the Infectious Disease Ward only. R.Wil. was a 19-year-old female student with moderately severe generalized paralysis. J.L. was a 21-year-old housewife with extreme paralysis of the shoulder girdle and upper arms. Case summaries are given in Appendix B. In these two pa-

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TABLE I

Time of institution and duration of oscillation and control intervals in eight patients with paralytic acute anterior poliomyelitis

Patient	Age	Sex	Control interval	Oscillation begun, day after onset of		Average daily period of oscillation	Duration of oscillation	Post-control interval reported
				Illness	Paralysis			
			days			*hours*	*days*	*days*
			Acute and early convalescent phase					
M. O'M.	21	F	4	8th	5th	14	33	30
J. C.	39	M	8	14th	12th	17	26	30
R. Wil.	19	F	3	7th	6th	10	17	28
J. L.	21	F	12	24th	24th	16	21	15
			Convalescent phase					
F. S.	20	M	18	53rd	50th	21	30	24*
			24*	107th	104th	21	18	30
C. R.	18	M	24	80th		†	30	18
				128th		‡	114	§
			Chronic phase					
J. W.	20	F	42	258th		18	42	21
R. V. K.	19	F	36	542nd		18	42	30

* Twenty-four-day interval between two intervals of oscillation.
† Sanders slow oscillation 14 hours daily, rapid rocking 7 to 8 hours daily.
‡ Rapid rocking only, 10 to 12 hours daily.
§ No post-control interval; patient began sitting in chair when rapid rocking was stopped.

tients daily 24-hour urine collections were made; stools were not collected. Their dietary intake was calculated but not weighed or kept rigidly constant since metabolic kitchen facilities were not available. The data of these two patients, therefore, are of less value than those of the other six patients. Dietary intakes of patients previously reported were given in Table I of the first paper (10). Dietary intakes of R.Wil. and J.L. as calculated from survey of food served and returned are given in Tables II and III of the present report.

Four patients were studied during the acute and early convalescent phases of poliomyelitis, two during the convalescent phase and two during the chronic phase. Duration of oscillation, time of its institution, length of control intervals before and after oscillation and other details of experimental procedure are given in Table I. Con-

TABLE II

Patient, R. Wil.—Average daily dietary calories, nitrogen and calcium; and urinary creatinine, nitrogen and calcium during acute and early convalescent phases of paralytic acute anterior poliomyelitis *

Day after onset	Dietary			Urinary			Phase of study
	Calories	N	Ca	Creatinine	N	Ca	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
4–6	1,142	12.4		0.950	15.01	0.132	Control
7, 8	1,055	12.2	0.661	1.042	16.80	0.206	Oscillating
10, 12	756	8.2	0.528	1.007	18.81	0.223	bed
13–15	938	10.0	0.810	1.037	17.72	0.221	
17–19	950	10.0	0.925	0.985	13.08	0.192	
20–23	1,080	11.5	0.925	0.863	13.59	0.211	
24, 25	1,085	10.8	0.912	0.860	12.29	0.159	Control
26, 27	1,176	11.6	0.912	0.875	13.39	0.157	
28–31	1,110	11.4	0.908	0.925	14.03	0.244	
32–34	1,185	12.3	0.958	0.900	14.00	0.218	
35–37	1,051	11.2	0.940	0.897	13.80	0.283	
39–41	1,124	11.9	0.941	0.850	14.52	0.274	
42, 43			0.900	0.775	12.38	0.240	
44–46	1,106	11.7	0.951	0.820	12.99	0.238	
47–49	1,085	11.2	0.892	0.760	11.98	0.237	
50, 51	953	9.3	0.883	0.760	10.92	0.265	

* Onset of illness, October 12, 1949; paralysis October 13.

trol intervals prior to oscillation were brief in the studies during acute and early convalescent phases but were from four to six metabolic balance periods (24 to 36 days) in duration in the observations during later phases.

When oscillation was begun, the bed was rocked for as many hours as possible of the 24, being stopped only for muscle re-education, meals, bed baths and use of the bedpan. Sleep was possible during oscillation and in some instances seemed to be aided by the motion. The daily period of oscillation averaged 19 hours for the patients studied during the convalescent and chronic phases, but was necessarily limited to 10 to 18 hours during the early weeks in two patients (M.O'M. and R.Wil.) because constant motion increased muscular discomfort, mainly around the shoulders. The bed mechanism was set to rock through the maximum arc, 18° to 21°, from horizontal (or 2° head-down) to extreme foot-down position and return in one and one-half minutes. The patient's feet were placed against a foot-board so that pressure was felt on soles and heels in the foot-down position.

One patient, C.R., was placed on a *rapid rocking bed* during part of his study to observe the influence of rapid rocking not on metabolism but on impaired ventilatory capacity. For a thirty-day period during the third and fourth months after onset of illness, he was placed on a rapid rocking bed for 7 to 8 hours a day (rate 16/min., total arc 24°, or 12° each side of horizontal) and on a Sanders bed for 14 hours daily. Later he remained on a rapid rocking bed for 10 to 12 hours a day for four months while other experimental procedures were carried out.

METHODS

Methods for analysis of urine, stool and serum for calcium, phosphorus, nitrogen, creatine, creatinine, sodium and potassium have been previously stated (10, 11). Methods of studying reaction of the circulation in motionless standing by tilt table test and of measuring girth of thighs and calves have also been previously described (10, 11). Coagulation time was determined by a modification (11) of the Lee and White method for venous blood and prothrombin time by the Link-Shapiro method (12).

Skin temperatures were measured periodically in four patients using the Hardy radiometer (13) which is more accurate and sensitive (± 0.01°C) than other devices and avoids errors inherent in the use of thermocouples placed directly on the skin (14). Readings were taken in 20 individual areas over the body surface; for calculation of the average skin temperature over the total surface of the body, readings were weighted by the formula of Hardy and DuBois (15). In two patients determinations of total peripheral blood flow to the skin were carried out by the method of Hardy and Soderstrom (16), as given in detail by Stewart and Evans (17) except that Hardy's original 20 areas of the skin were used. In addition to measurements of skin temperature with the radiometer, the total peripheral blood flow method makes use of measurements of rectal temperature with a single junction copper-constantan thermocouple (± 0.02°C) (13) and of oxygen consumption with a Benedict-Roth metabolism apparatus (18). All determinations of skin temperature and peripheral blood flow were made in the basal metabolic state in a controlled temperature room; for each determination at least two sets of skin temperature readings were made at intervals of 20 minutes.

RESULTS

A. *Metabolic Constituents*

1. *Calcium excretion and balance.* In the four patients studied during the acute and early convalescent phases, urinary calcium was increasing gradually during the brief control interval prior to oscillation. When oscillation was begun, a variety of responses took place. In one patient, J.C., urinary calcium continued to rise for another six to nine days when a level averaging 0.534 gm. per day was reached, then maintained for several weeks (Figure 1). In M.O'M., coincident with beginning of oscillation, urinary calcium remained at a level of 0.254 gm. per day for two weeks; then, while the patient was still on the oscillating bed, urinary calcium rose to a new level of 0.370 gm. per day (Figure 1). R.Wil.'s urinary calcium remained quite constant during oscillation at 0.210 gm. per day (Figure 2). In the fourth patient, J.L., who with R.Wil. was not on the Metabolism Ward, urinary calcium declined from an average level of 0.394 gm. per day at the time oscillation was instituted to 0.313 gm. In the two patients on whom balance studies had been begun, J.C. and M.O'M., calcium balances were − 0.385 and − 0.295 gm. per day, respectively, during oscillation on a constant calcium intake of 0.920 gm. per day (Figure 1).

Since it was difficult to compare the changing levels of excretion of the brief initial control phases with those during oscillation and also since dietary calcium intakes were varying until after oscillation had been started, particular attention was focused on whether calcium excretion would rise when oscillation was discontinued. When that was done, three of the four patients showed no change in urinary calcium excretion; in the two on complete balance studies, no significant change occurred in negative calcium balance. In the fourth patient, R.Wil., mean daily urinary calcium excretion increased from 0.210 gm. per day to an average of

TABLE III

Patient, J. L.—Average daily dietary calories, nitrogen and calcium; and urinary creatinine, nitrogen and calcium during early convalescent phase of paralytic acute anterior poliomyelitis *

Day after onset	Dietary			Urinary			Phase of study
	Calories	N	Ca	Creatinine	N	Ca	
		gm.	gm.	gm.	gm.	gm.	
12–14	1,182	6.7	0.315	0.800	12.32	0.270	Control
15–17	1,787	9.8	0.648	0.897	13.82	0.296	
18–20	1,895	9.6	0.465	0.856	12.68	0.362	
21–23	1,705	9.2	0.485	0.747	9.68	0.381	
24–26	1,732	9.0	0.365	0.803	12.76	0.407	Oscillating bed
27–29	2,000	9.1	0.602	0.750	11.70	0.315	
31, 32	1,970	7.8	0.359	0.720	9.33	0.332	
33–35	2,293	10.1	0.643	0.720	10.25	0.316	
36–38	1,741	10.7	0.595	0.817	12.67	0.285	
39–41	2,068	8.2	0.927	0.690	9.75	0.309	
42–44	2,106	8.8	0.805	0.707	11.37	0.319	
45–47	2,413	10.9	1.119	0.640	10.13	0.302	Control
48–50	2,118	11.7	0.892	0.656	10.01	0.308	
51–53	2,258	10.4	0.997	0.750	10.72	0.330	
54–56	1,944	11.2	0.812	0.677	9.96	0.284	
57–59	1,865	11.0	0.834	0.690	11.61	0.329	

* Onset of illness and paralysis, October 1, 1948.

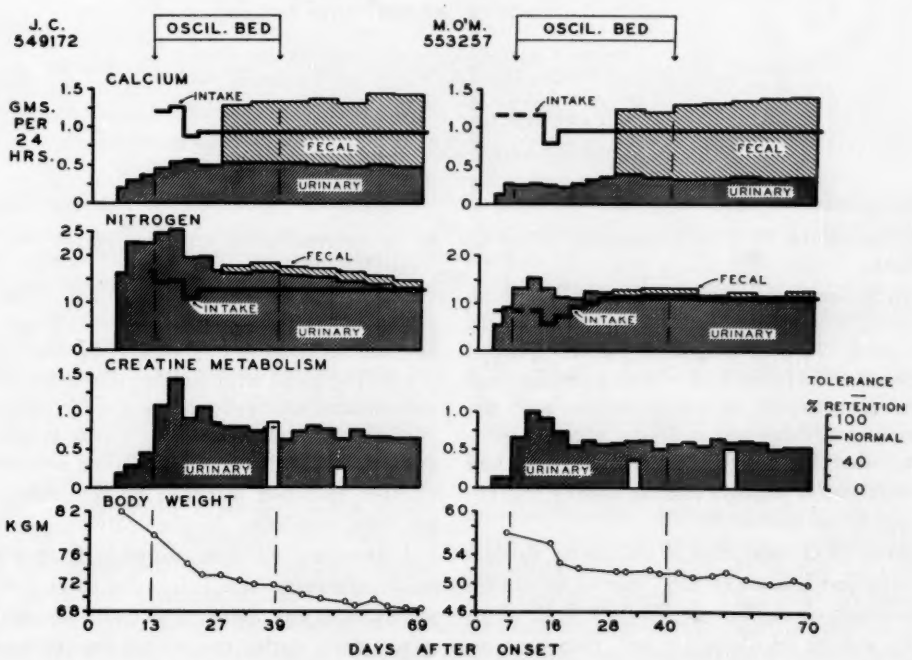

FIG. 1. CALCIUM AND NITROGEN BALANCE, URINARY CREATINE, CREATINE TOLERANCE AND BODY WEIGHT PRIOR TO, DURING AND FOLLOWING OSCILLATION IN THE ACUTE AND EARLY CONVALESCENT PHASE, PATIENTS J.C., 39-YEAR-OLD MALE AND M.O'M., 21-YEAR-OLD FEMALE

Calcium and nitrogen data are charted with intake and output both plotted upward from the base-line and urinary output on the bottom in order to portray clearly changes in urinary excretion of these elements as well as changes in balance.

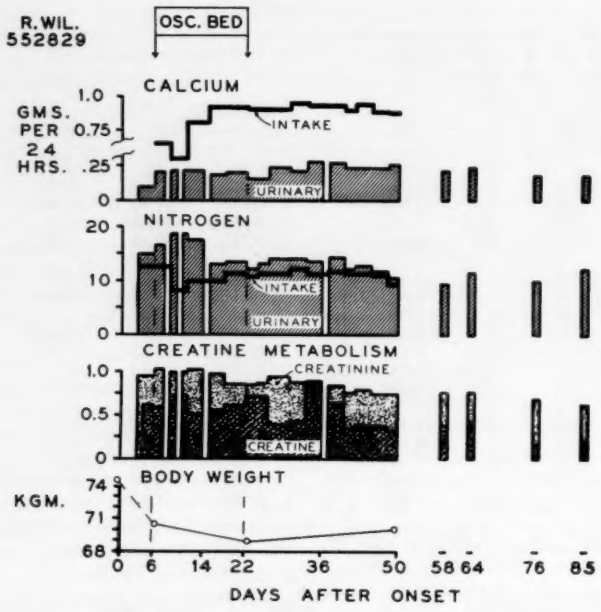

FIG. 2. URINARY CALCIUM, NITROGEN, CREATINE AND CREATININE AND BODY WEIGHT PRIOR TO, DURING AND FOLLOWING OSCILLATION IN THE ACUTE AND EARLY CONVALESCENT PHASE, PATIENT R.WIL., 19-YEAR-OLD FEMALE

Urinary creatine and creatinine are plotted together, creatinine in stippled tone overlapped by creatine in cross-hatched tone.

0.250 during the 28 days immediately following oscillation.

Two patients, F.S. and C.R., were placed on oscillating beds during the third and fourth months after onset. In F.S. (Figure 3), a very gradual downward trend in the level of urinary calcium and decreasing negativity of calcium balance were unaffected by a 30-day and an 18-day phase of oscillation. In C.R., a high level of urinary calcium excretion and of negative calcium balance was unchanged by oscillation.

During the chronic phase of the disease, calcium excretion and balance of J.W. showed no change as the result of oscillation. While R.V.K. (Figure 4) was on an oscillating bed, there was reduction in calcium excretion, mainly in the fecal component, and development of a positive calcium balance (+ 0.209 gm. per day). This change probably should not be attributed to oscillation, however, since calcium excretion did not revert to its original level after oscillation was discontinued.

2. *Nitrogen excretion and balance.* The only suggestive influence of oscillation on nitrogen metabolism occurred in F.S. during the convalescent phase (Figure 3) when urinary nitrogen excretion and negative nitrogen balance were gradually diminishing. Oscillation did not appear to alter this gradual decline, but when oscillation was stopped nitrogen excretion increased by approximately 1 gm. daily, suggesting the possibility that oscillation had decreased nitrogen excretion from what it would otherwise have been. To check this possibility, F.S. was placed on an oscillating bed for a second time; during this second observation average nitrogen excretion was not significantly decreased, and no after-rise took place when oscillation was discontinued. The reason for the change in nitrogen excretion following the first phase of oscillation is obscure. No changes in

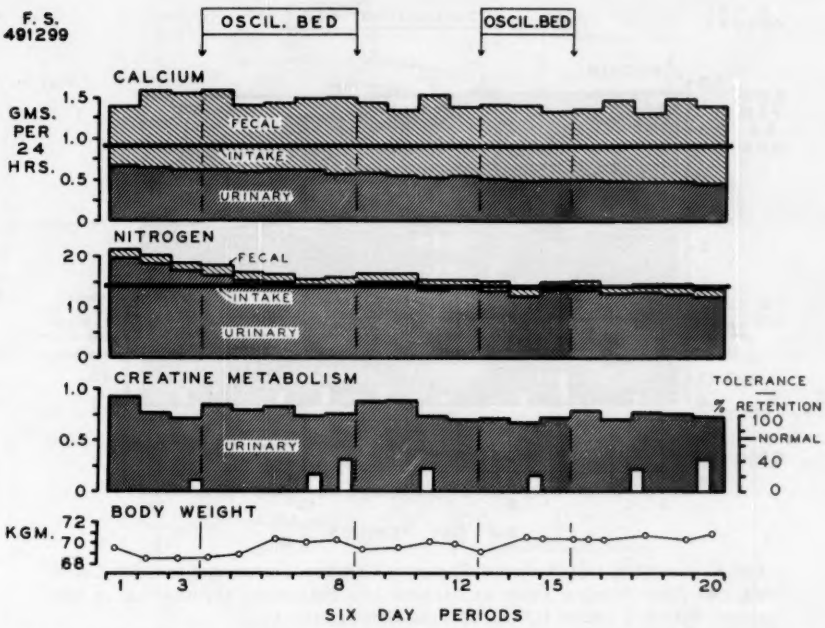

FIG. 3. CALCIUM AND NITROGEN BALANCE, URINARY CREATINE, CREATINE TOLERANCE AND BODY WEIGHT PRIOR TO, DURING AND FOLLOWING A 30-DAY AND AN 18-DAY PERIOD OF OSCILLATION IN THE CONVALESCENT PHASE, PATIENT F.S., 20-YEAR-OLD MALE

Explanation of construction of balance chart for calcium and nitrogen in legend for Figure 1.

nitrogen excretion attributable to oscillation occurred in any of the other patients.

Although in J.C. and M.O'M. urinary nitrogen was at its highest level during their first twelve days on the bed, this finding was thought not attributable to oscillation since the same pattern of nitrogen excretion was seen during the same period after onset of illness in two other patients, D.Q. and D.A., who were not placed on oscillating beds.

3. *Other metabolic constituents.* In one patient, M.O'M., urinary citric acid increased with the rise in urinary calcium during the period she was on an oscillating bed. Since, however, this patient's citric acid excretion remained high after oscillation was discontinued, the elevation in citric acid was thought not to be an effect of oscillation.

In various patients minor changes occurred in urinary creatine excretion, creatine tolerance and in sodium balance which had no clear or consistent relationship to oscillation. No influence of oscillation was noted in phosphorus and potassium excretion and balance, in urinary creatinine and in serum calcium, phosphorus and alkaline phosphatase.

The urinary calcium and nitrogen data of patients R.Wil. and J.L. are given in Tables II and III. Data of patients J.C., M.O'M., F.S., C.R., J.W. and R.V.K. are given in tables in Appendix A.

B. *Physiological Measurements*

1. *Circulatory reactions to gravity as measured by tilt table tests.* During initial periods of study when the patients were largely confined to bed, regardless of phase of poliomyelitis, all patients exhibited impairment in the ability to maintain adequate circulation in the upright position. This impairment was indicated by the development of dizziness, pallor, darkening of vision, and slight nausea within thirty minutes of the time patients were elevated to an angle of 40 to 50 degrees from the horizontal on a tilt table. Impairment was more marked during the early convalescent phase

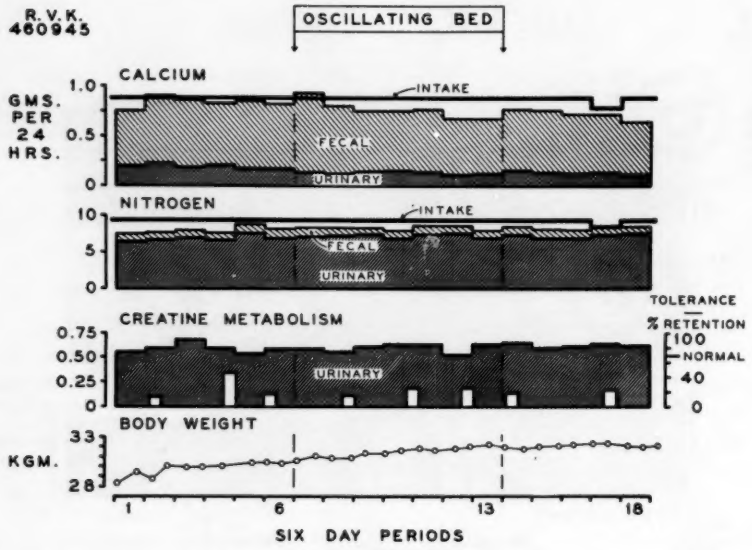

FIG. 4. CALCIUM AND NITROGEN BALANCE, URINARY CREATINE, CREATINE TOLERANCE AND BODY WEIGHT PRIOR TO, DURING AND FOLLOWING OSCILLATION IN THE CHRONIC PHASE, PATIENT R.V.K., 19-YEAR-OLD FEMALE

Explanation of construction of balance chart for calcium and nitrogen in legend for Figure 1.

of the disease, symptoms appearing in less than twenty minutes when patients were tested on the tilt table during this phase.

A series of tests were performed at intervals before, during and following the periods of study on the oscillating bed, on patients F.S. and C.R. during the convalescent phase and on J.W. and R.V.K. during the chronic phase. These tests showed that during the oscillation phase patients could tolerate a longer period in the upright tilted position before symptoms heralding fainting began to occur. Figure 5 indicates the effect of oscillation on tilt table tests in these patients, showing the number of minutes required for the pulse pressure to fall to a level of 10 to 12 mm. of mercury when the subjects were tilted upright on the tilt table. This pulse pressure level was selected as the end-point for comparison of various tilt table tests as a result of analysis of tests performed during the immobilization study of normal subjects. Correlation of data on blood pressure and pulse rate with symptomatic reactions had indicated that pulse pressure was the most important factor involved in the response of the circulation to tilting. With a subject in the upright position on the tilt table, it was found that when the pulse pressure became reduced to between 10 and 12 mm. of mercury a critical level was reached at which circulation became impaired, dizziness and pallor appeared and fainting followed shortly thereafter. The next most important factor was the fall in systolic pressure. Similarly, Graybiel and McFarland (19), in an analysis of tilt table tests on ninety-one normal individuals, concluded that pulse pressure and fall in systolic pressure were the most important factors indicating a failing circulation.

2. Skin temperature and peripheral blood flow. A number of studies of skin temperature and peripheral blood flow were carried out on the two patients observed during the chronic phase of the disease, seven on J.W. and three on R.V.K.; both patients had marked paralysis of the leg muscles and clinically "cold" lower extremities.

Oscillation over a period of six weeks brought about no increase in the average skin temperature of the whole body (measured in 20 areas), but significantly raised the skin temperature of the

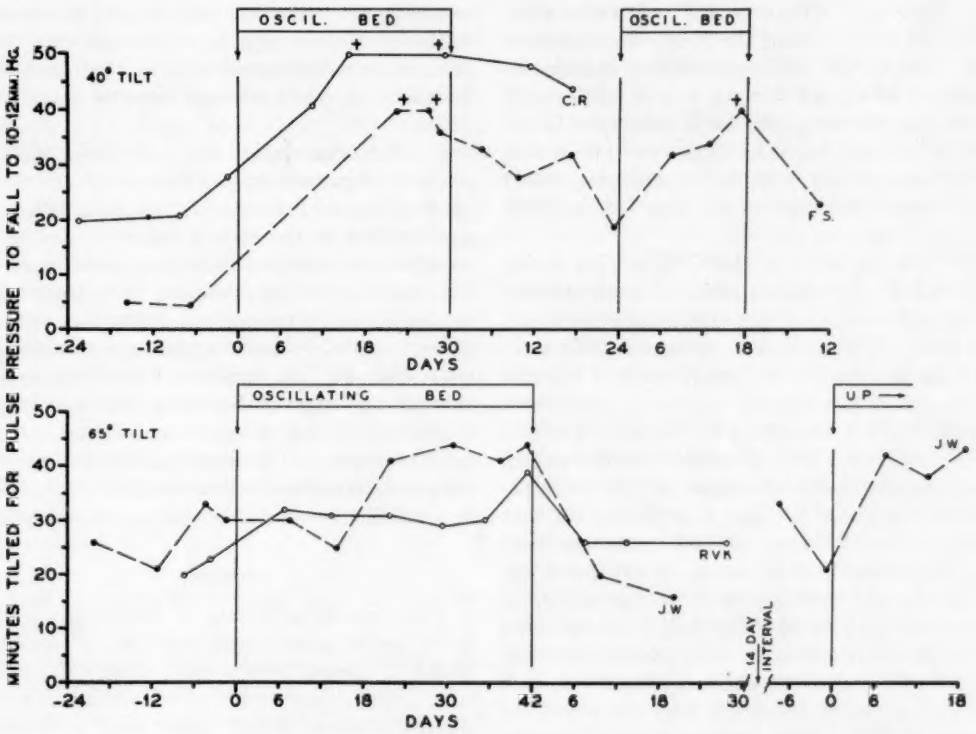

FIG. 5. EFFECT OF OSCILLATION ON THE RESULTS OF TILT TABLE TESTS IN FOUR PATIENTS DURING THE CONVALESCENT (UPPER PART OF GRAPH) AND CHRONIC (LOWER) PHASES, SHOWING THE NUMBER OF MINUTES IN THE TILTED POSITION FOR THE PULSE PRESSURE TO FALL TO CRITICAL LEVELS OF 10 TO 12 MM. OF HG

Plus sign beside test result indicates patient was able to remain in the tilted position for longer than the time shown without developing critical level of pulse pressure.

feet. In J.W. prior to oscillation, feet skin temperatures (average of two areas) were 34.0 and 33.9 degrees C in measurements on two occasions. On the first study during oscillation no appreciable change was noted, but readings in the next three studies were 35.6, 35.8 and 35.45 degrees C (bed stationary during tests). Two weeks after oscillation was discontinued the reading was 35.0 degrees C. Room temperatures in the climatically controlled room during the tests were within ± 0.5 of 30.0 degrees C, averaging 30.0 before, 30.2 during, and 30.0 after oscillation. In R.V.K., feet skin temperature was 30.0 degrees C at a room temperature of 29.3 in a single determination (two sets of readings) before oscillation was begun. During the oscillation phase feet skin temperature rose to 33.8 and 33.9 degrees C; room temperatures were 29.3 and 30.2 degres C, respectively. Leg temperatures (four areas) of R.V.K. increased from 33.0 to 34.5 and 34.0 degrees C, but in J.W. the mean leg temperature increase was only 0.4 degrees C. Measurements of feet skin temperature in both patients first with the oscillating bed stationary and then while the bed was oscillating revealed no acute warming effect as measured either within a few minutes after the bed was started or after the bed had been oscillating for 40 to 90 minutes.

Peripheral blood flow determinations yielded results which were not clear inasmuch as there seemed to be an acute effect of oscillation in one patient and a chronic effect in the other. Two determinations in J.W. before oscillation were 158 and 184 ml. per M.^{2} per min., mean 171; during oscillation successive values were 165, 177, 173 and 176 ml. per M.^{2} per min. (bed stationary dur-

ing tests) with a value of 166 two weeks after. With the bed oscillating for 90 minutes, values of 199, 214, 165 and 231 were obtained, a mean increase of 30 ml. per M.^{2} per min. or 17 per cent. In R.V.K. the peripheral blood flow was 114 ml. per M.^{2} per min. before oscillation and 144 on each of two tests during; with the bed oscillating, blood flow further increased to 151 and 153, an additional 5.5 per cent rise.

In two patients, F.S. and C.R., studied during the convalescent phase a series of seven and five tests, respectively, of skin temperature were carried out. With oscillation, changes in total average skin temperature and temperature of legs and feet were all less than 0.7 degrees C. Peripheral blood flow was not determined in these patients.

3. *Leg girth.* No consistent effect on leg girth was brought about by oscillation. In three patients, C.R., R.V.K., and F.S. during his first study on the oscillating bed, an increase was noted in circumference of the calves of 1.0 to 1.3 cm. This increase was thought to be due mainly to accumulation of extracellular fluid in the legs from sustained increased hydrostatic pressure resulting from the fact that the bed was adjusted to rock back just to the horizontal from the foot-down position. This retention of fluid in leg tissues was manifested by slight puffiness about the ankles in F.S. and must partially account for the weight gain during oscillation in this patient (Figure 3). In patients for whom the bed was adjusted to rock back to approximately 2 degrees head down, permitting leg veins to empty fully, increase in leg girth was either slight or did not occur; in F.S.'s second time on the bed with the bed so adjusted, for example, increase in leg girth was minimal.

4. *Ventilation.* One patient in the chronic phase of poliomyelitis, R.V.K., had a markedly limited vital capacity, 505 ml. During six weeks of oscillation her vital capacity increased gradually to 640 ml.

F.S., placed on an oscillating bed two months after onset, showed an increase in vital capacity with oscillation from 3400 ml. to 3680 ml. and an increase in maximum minute ventilation capacity from 60 to 74 liters; during control periods after oscillation these values did not increase further. C.R., also in the convalescent phase, because of limited ventilatory capacity was placed on a rapid rocking bed as well as on a Sanders bed. Increases in vital capacity, tidal air and maximum minute ventilation capacity in this patient were presumably mainly attributable to rapid rocking, the effects of which we have reported elsewhere (20).

5. *Other physiological tests.* Periodic ergometer tests of gastrocnemius-soleus muscle power in two convalescent patients, F.S. and C.R., indicated no alteration in the rate of recovery of muscle strength as the result of oscillation. Basal metabolism was measured approximately every two weeks in six patients (in two patients during each disease phase), and no influence of oscillation was noted; tests while the bed oscillated showed the cyclic shift in diaphragm mid-position level previously mentioned (1) but no significant increase in oxygen consumption. Clotting and prothrombin times remained normal in the three patients (F.S., C.R. and R.V.K.) in whom this function was measured.

COMMENT

These studies of effects of the Sanders oscillating bed in patients with paralytic poliomyelitis indicated modest physiological changes from its use. These changes were not of the kind or magnitude, however, which would lead to recommendation of the bed for management of patients immobilized by poliomyelitis.

Changes in calcium excretion

The metabolic function under particular scrutiny was calcium excretion and balance. Since losses of calcium in paralytic poliomyelitis are sufficient to lead to osteoporosis and possibly to urinary tract stone formation (21), a means of diminishing these losses would be of considerable value. During acute and early convalescent phases, when demonstration of a restraining action on calcium excretion would have been particularly meaningful, preliminary control periods were necessarily brief and calcium intake variable so that comparison of initial levels of calcium excretion with levels during the oscillation periods was difficult to interpret. Positive effect of oscillation, therefore, would be indicated only by a significant rise in calcium excretion after oscillation had been stopped. In only one of the four patients studied during this early phase was the mean urinary calcium excretion after oscillation statistically different from

the mean level during oscillation; the difference between these means in R.Wil. was 3.5 times the S.E. of the difference between the means (22). However, although this difference was *statistically* significant, a change of much greater magnitude would be necessary for one to consider that oscillation had prevented calcium loss to a *biologically* significant degree. Furthermore, even if oscillation had been shown to minimize calcium losses during the early phases, muscle spasm, tenderness and discomfort during the early weeks were in a number of patients accentuated by oscillation, suggesting impracticality. Studies during the later convalescent and chronic phases indicated no appreciable reductive action of oscillation on calcium loss. These largely negative findings with respect to the effect of oscillation on calcium metabolism are in general agreement with the previously mentioned results of Wyse and Pattee in four paraplegics (9).

Possible factors in lack of effect on calcium excretion

A number of reasons may be suggested for the ineffectiveness of the oscillating bed in preventing calcium loss in paralytic poliomyelitis as compared with its benefit in immobilized normal subjects. In the latter investigation oscillation was begun as soon as the subjects were placed in casts. Although in the poliomyelitis patients observed during the acute phase the duration of paralysis prior to oscillation was only a few days (mean for first three patients, 8 days), it is conceivable that a trend toward calcium loss may have already become so well established that the oscillating bed could not appreciably reverse the process. A second difference between the two studies is that, despite the fact that the normal subjects were in casts from waist to toes, the degree of immobilization of the poliomyelitis patients was greater. Furthermore, neurological injury *per se* may have had some influence on calcium balance, although evidence has been previously cited (21) that this was probably not a major factor and that immobilization seemed principally responsible for calcium loss in these patients.

Mechanical factors may also have been partly responsible for differences in the two series. Muscle paralysis in the poliomyelitis patients either greatly reduced or prevented muscle pull on periosteal surfaces of the leg bones; in the normal group such a pull was thought to occur to a considerable degree as the bed shifted its position back and forth. Since this form of "stress and strain" was largely absent in the poliomyelitis patients, stimulation of bone formation was limited to the slight, intermittent weight-bearing occurring in the foot-down position of the bed. This was far less than *full* weight-bearing, an apparently potent stimulus to mineral storage even in the absence of leg muscle function, as demonstrated by the Abramson study (23) of ambulatory paraplegics, a different type of paralysis from poliomyelitis.

Another possible mode of action of oscillation postulated to explain the favorable result in the experiment with normal subjects (1) was an increase in peripheral and, presumably, bone circulation. As mentioned in the preceding paper, however, there is no agreement that bone formation is favored by increased circulation. Further, although in both studies the bed's motion was identical and improved circulation to the legs and feet was indicated, the definite effect on calcium excretion observed in the normal group was not seen in the poliomyelitis study. With this evidence, it may be questioned whether circulatory effects of the oscillating bed had any relationship to the favorable effects with respect to calcium in the normal subjects.

Reaction of circulation to gravity

Among the various physiological functions, the mechanisms responsible for circulatory control in the upright position have been the most consistently influenced by oscillation. In immobilized normal subjects circulatory reflexes as measured by tilt table tests were maintained almost at control levels by oscillation and their deterioration from immobilization on a fixed bed largely prevented (1). Wyse and Pattee (9) reported the bed to be of value in restoring vasomotor tone of the extremities in their paraplegic patients. In the present study when patients were being oscillated, they could tolerate a much longer period of time in the upright position on the tilt table.

The degree of impairment of these circulatory reflexes in paralytic poliomyelitis appeared to be

similar to that resulting from immobilization alone. The altered tilt table test response of pulse and blood pressure among the five patients tested (before and after oscillation) was of approximately the same magnitude as was noted in the normal subjects immobilized on a fixed bed for six to seven weeks (11). Analysis of the measurements of the circumference of the legs made every two to three minutes during and after tilting in tests during the study with normal subjects had suggested that during immobilization there was impairment of either venous or muscular tone or both. Similar measurements in two poliomyelitis patients studied during the chronic phase revealed no differences in the changes in these measurements from those in the normal subjects. No evidence was elicited in the patients to indicate that muscle paralysis due to poliomyelitis resulted in poorer support of blood vessels than would occur from immobilization alone. The possibility of a circulatory effect of paralysis of this sort cannot be excluded, however, in view of the small number of tests made with these measurements.

Peripheral circulatory effects of oscillation

Beneficial results observed in vascular diseases of the lower extremities with use of the oscillating bed have been attributed to improvement in blood flow brought about by the motion of the bed. Conclusive evidence for this action has not been presented although some measurements of peripheral circulation have been made (6, 24). In the study of normal subjects (1) change in venous pressure in the dorsal footveins of 140 mm. of water with each cycle of the bed was interpreted to indicate that in the presence of competent venous valves a larger volume of blood was being delivered to the right auricle during oscillation; skin temperatures were not measured. In the present study, although no *immediate* warming effect of skin temperatures of the feet and legs by oscillation was demonstrated, over the course of three weeks a distinct increase in feet skin temperature was noted in the two patients studied during the chronic phase. An increase of more than 1°C measured with the Hardy radiometer can be considered highly significant, particularly under the environmental conditions used for these measurements. The high room temperature (30°C) favored near-maximal vasodilatation, and the fact that these tests were done during winter months reduced variations in relative humidity, so that environmental influences on circulation were minimal and relatively constant. The increase in feet skin temperature was particularly marked in the patient who evidently had the greater impairment of peripheral circulation; in this patient a significant increase in leg skin temperature from oscillation was also noted. In two patients studied during the convalescent phase when peripheral circulation was not clearly impaired, skin temperatures of the legs and feet were not significantly raised by oscillation. These results suggest that an effect of oscillation on lower extremity skin temperature can be more readily demonstrated if the circulation is already impaired when oscillation is begun.

Results of determinations of the effect of oscillation on peripheral blood flow over the area of the whole body were not conclusive. No explanation is available for the differing results of an acute increase in blood flow from oscillation in one patient and a chronic similar effect in another.

Basal metabolism and ventilation

Tests of basal metabolic rate in normal subjects (1) had shown that oscillation in the Sanders bed did not increase oxygen consumption over that in the resting horizontal position. This finding was confirmed in the present studies on patients with poliomyelitis.

During basal metabolism tests on the normal subjects with the bed oscillating, the metabolism chart indicated periodic shifting of the diaphragm's mid-position synchronous with shifting of the bed from horizontal to foot-down position and return. At this slow rate of rocking one would not expect an increase in resting pulmonary ventilation per minute, and none was noted in either study. However, two poliomyelitis patients with sub-normal vital capacities showed modest increases in this measurement during four to six weeks of oscillation which did not continue after oscillation was stopped, and one of these patients showed a 23 per cent increase in maximum minute ventilation capacity. These suggested influences of oscillation on ventilation led to an interest in the possible effects of rocking at a rapid rate which could be

synchronized with respiration (25); these latter studies have been reported elsewhere (20).

The present study was not intended to accumulate data which could lead to evaluation of the metabolic effects of rapid rocking, now frequently used in the management of poliomyelitis cases with respiratory involvement, particularly in weaning patients from tank respirators. Rapid rocking was used during part of this study in one patient, and no unusual or significant findings were observed except for increases in vital capacity, tidal air and maximum minute ventilation capacity.

Clinical use of tilt table

No studies were carried out of the possible clinical usefulness of the tilt table. This has been advocated for the purpose of lessening "the tendency to renal stones" (26) although data supporting the suggestion were not cited. Frequent use of the table might improve vasomotor tone, but the impairment of mechanisms responsible for circulatory control in the upright position seen in the present study suggests that caution should be exercised in the table's use because of the danger of fainting. The possible metabolic derangements and dangers of undue muscle stretching in too early weight-bearing in this disease (27) were mentioned in the preceding report (21).

Clinical application of oscillating bed

The present generally negative report indicates that the oscillating bed would not be useful for modifying the metabolic derangements of paralytic poliomyelitis. The possibility remains that the bed would have such value in other immobilizing illnesses if applied at their inception. Wyse and Pattee did not employ the oscillating bed in paraplegia until more than one month after paralysis had occurred by which time oscillation was ineffective except for improvement in vasomotor tone.

Beneficial circulatory effects, seen also in both of our studies, suggest the likelihood that the oscillating bed may have more valid use as an aid when ambulation is to be instituted, particularly after several weeks in bed following severe trauma, a cerebro-vascular accident, paraplegia or paralysis due to poliomyelitis. Usefulness of the bed in this respect was recently demonstrated in the rehabilitation of an associate who had suffered a crippling stroke. Suggestion for this use of the bed came from the observation in healthy subjects that restoration of metabolic and physiological indices to normal came about much more rapidly following immobilization in the oscillating than in the fixed bed (1).

The present study appears to have raised or left unsettled several questions. It seems evident, however, that some means other than the oscillating bed is needed for reduction of calcium loss in paralytic poliomyelitis. One such means will be suggested in the succeeding report.

SUMMARY

This third paper of a series on studies in paralytic acute anterior poliomyelitis has described the results of a trial of the slowly oscillating bed in eight patients; four patients were placed on the bed during the acute and early convalescent phase, two during the convalescent phase and two during the chronic phase. Patients were oscillated for periods of 17 to 42 days, the daily period of oscillation averaging 17 hours. The principal findings were as follows:

1. Oscillation did not retard the natural increase in calcium excretion occurring during the acute and early convalescent phases. During the convalescent and chronic phases oscillation exerted no reductive action on negative calcium balance. Possible reasons were suggested for the difference in result between this study and one in which oscillation diminished calcium loss in immobilized normal subjects.

2. No certain effect of oscillation was noted on nitrogen excretion and balance, phosphorus, potassium and sodium balance, urinary creatine, creatinine and citric acid or on serum calcium, phosphorus and alkaline phosphatase.

3. In all phases of the disease if the patients were largely confined to bed, they exhibited impairment in the mechanisms essential for adequate circulation in the erect position, as measured by tilt table tests. During the periods on an oscillating bed patients demonstrated definite improvement in these mechanisms, being able to tolerate a longer period of time in the upright tilted position before reaching critically low levels of pulse pressure.

4. Skin temperature of the feet rose during several weeks on the oscillating bed in two patients with impaired peripheral circulation in the chronic phase of the disease. In two patients in the convalescent phase, oscillation brought about no change in skin temperature.

5. Among other physiological measurements, no definite effect of oscillation was noted on leg girth, muscle strength or basal metabolism.

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METABOLIC STUDIES IN PARALYTIC ACUTE ANTERIOR POLIOMYELITIS. IV. EFFECTS OF TESTOSTERONE PROPIONATE AND ESTRADIOL BENZOATE ON CALCIUM, PHOSPHORUS, NITROGEN, CREATINE AND ELECTROLYTE METABOLISM [1]

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WITH THE TECHNICAL ASSISTANCE
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The first two papers in this series (1, 2) described various marked metabolic derangements resulting from paralytic acute anterior poliomyelitis, particularly those involving nitrogen, creatine, calcium and phosphorus metabolism. The third paper (3) related the results of an attempt to minimize these extensive derangements by use of the slowly oscillating bed. The present paper reports results of an effort to modify the disordered metabolism of paralytic poliomyelitis by means of gonadal steroid hormones. These steroids have been shown in various clinical states to be effective in reducing the excretion of nitrogen, calcium and phosphorus and in favoring the positive balance of these elements. Because of these effects, it seemed reasonable to investigate whether or not gonadal steroids would act in a similar manner in a disease following which development of muscle and bone atrophy is prominent.

More specifically, the suggestion that gonadal steroids might have value in paralytic poliomyelitis arose from a variety of studies and observations. The protein anabolic effects of androgens have been summarized by Kochakian (4). Papanicolaou and Falk (5) were evidently the first to show that testosterone administration would bring about hypertrophy of the general musculature, indicating that the retained protein was stored in muscle. The effects of androgens on calcium metabolism in post-menopausal and senile osteoporosis have been summarized by Albright and Reifenstein (6, 7). They and others (8–11) have shown the effectiveness of androgens in the osteoporosis of Cushing's syndrome. Presumably androgens act by increasing the amount of protein osteoid matrix, essential for bone formation. Estrogen administration has been shown to be useful in postmenopausal osteoporosis (12, 13), in the osteoporosis of acromegaly (14) and in osteoporosis associated with Paget's disease (15). Less effective than androgens in enhancing protein deposition, estrogens are thought to act by stimulating osteoblasts to lay down more protein matrix.

The effects of both types of gonadal hormones on the skeleton of animals have been extensively studied and summarized by Gardner and Pfeiffer (16). A study particularly related to the present one is that of Armstrong, Knowlton, and Gouze (17) in which disuse atrophy of the skeleton was brought about in mature rats by severing the brachial plexus; estradiol in modest doses was found generally to prevent the atrophy whereas testosterone did not, although only very large doses of the latter steroid were given.

PROCEDURE

The general plan, procedure, clinical care and diets employed in these studies were outlined in the first paper in this series (1). The present report is based on the data of seven patients (D.A., J.C., R.W., F.S., C.R., J.W. and R.V.K.), all of whom were studied on the Metabolism Ward where closely controlled balance studies were carried out. J.W. and R.V.K. were females and the others males. Changes in diet, physical therapy or activity of the patients were in nearly all instances made not less than two weeks prior to and two to four weeks following a course of androgen or estrogen in order to provide suitable control periods before and after the administration of these hormonal agents. The relatively slow progress of physical improvement in these extensively paralyzed patients made this control possible.

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

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† Died, January 6, 1956.

The hormonal preparations used were testosterone propionate, given intramuscularly in doses of 25 and 50 mgm. daily, and alpha-estradiol benzoate (Progynon-B), given intramuscularly in doses of 1.66 and 3.32 mgm. (10,000 and 20,000 rat units) daily. Testosterone propionate alone was given during various phases of the disease in eight studies for periods ranging from 12 to 42 days. Estradiol benzoate was given alone in two studies during the convalescent phase, for 18 and 30 days each. The two hormones were given concurrently (in separate syringes) in six studies for periods of 12 to 36 days. Four patients were given combined testosterone and estradiol at one time and testosterone alone at another so that in their data comparison was possible of

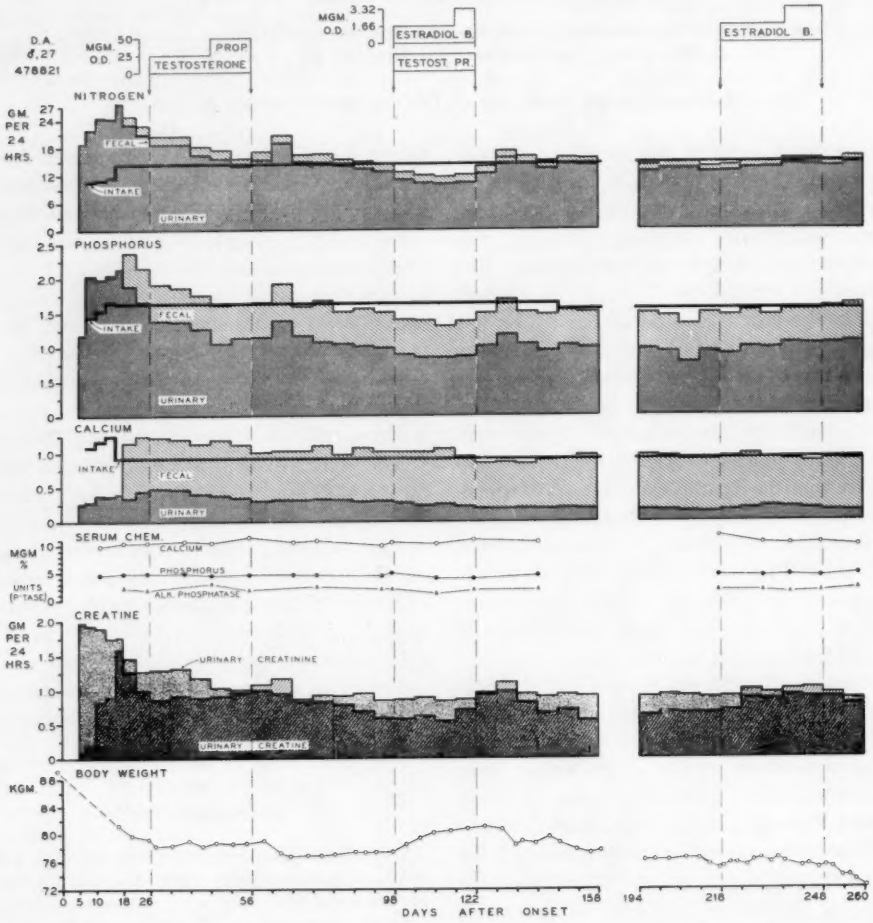

FIG. 1. METABOLIC DATA ON PATIENT D.A., 27-YEAR-OLD MALE, DURING THE FIRST NINE MONTHS AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE, COMBINED STEROID AND ESTRADIOL BENZOATE GIVEN DURING THE EARLY, MID AND LATE CONVALESCENT PHASES, RESPECTIVELY

The nitrogen, phosphorus and calcium data are charted with the intake and output both plotted upward from the base-line and urinary output on the bottom in order to portray clearly the changes in the urinary excretion of these elements as well as the changes in balance. The ordinate scales for nitrogen and phosphorus are arranged in the ratio of 1 gram of phosphorus to 15 grams of nitrogen; the ordinate scales of calcium and phosphorus are arranged in the ratio of 1:1. Urinary creatine and creatinine are plotted together, the creatinine in stippled tone overlapped by creatine in cross-hatched tone.

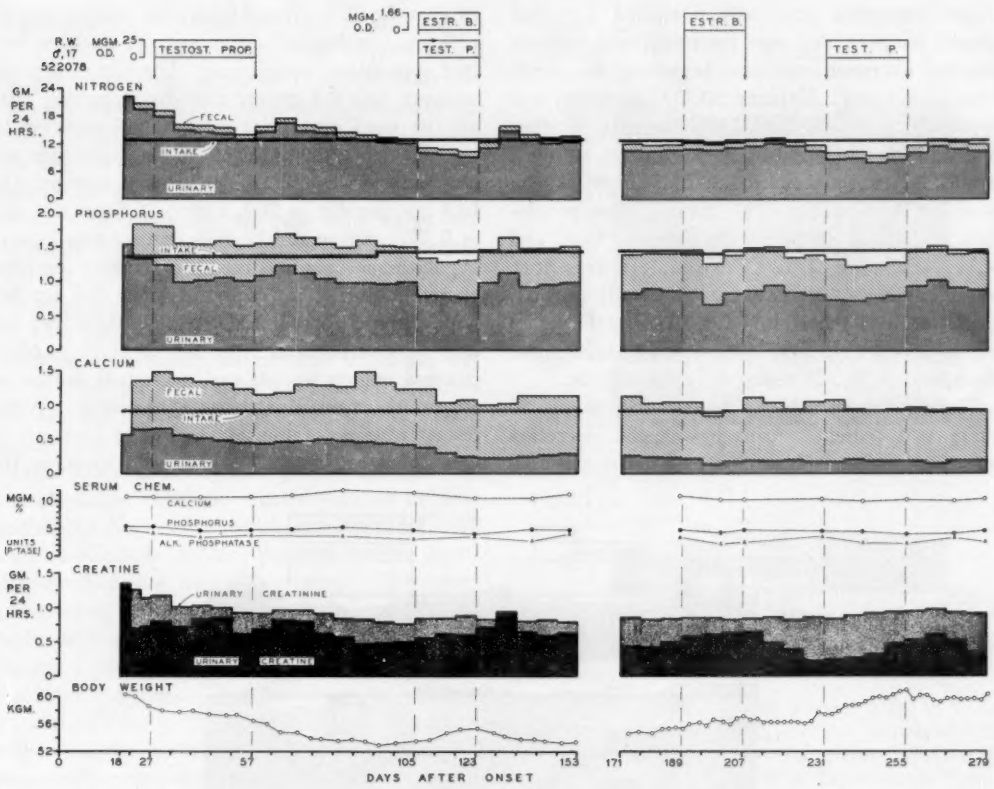

FIG. 2. METABOLIC DATA ON PATIENT R.W., 17-YEAR-OLD MALE, DURING THE FIRST NINE MONTHS AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE GIVEN DURING THE EARLY CONVALESCENT AND CHRONIC PHASES, AND OF COMBINED STEROID AND ESTRADIOL BENZOATE GIVEN DURING THE CONVALESCENT PHASE

Explanation of construction of chart is in legend for Figure 1.

combined administration of the hormones with those of androgen alone.

METHODS

The methods for analysis of urine, stool and serum for the various chemical constituents have been given in the first two papers of this series (1, 2). Metabolic balance studies were carried out in six-day periods. The methods for measuring girth of thighs and calves and strength of muscle groups were given in the first paper (1). Tests of basal metabolic rate were performed every 10 to 14 days by the method of Benedict and Roth (18). Measurements of skin temperature were made in two patients (F.S. and C.R.) by the method described in the third paper of this series (3).

Definitions and abbreviations. In this paper the various phases of illness are defined as follows: early convalescent, from the third to eighth week after onset of illness; convalescent, second through sixth month; and chronic, after the sixth month. The following abbreviations are employed: t.p. = testosterone propionate; e.b. = estradiol benzoate; and in Table III, Comb. = Combined testosterone propionate-estradiol benzoate administration.

RESULTS

I. *Observations of Metabolic Constituents*

A. *Effects on nitrogen, creatine, glycocyamine, body weight, calcium, phosphorus, citric acid, 17-ketosteroids and serum calcium, phosphorus and alkaline phosphatase*

1. *Effects of testosterone propionate administration.*

a. Early convalescent phase. By the fourth week after onset of illness the high urinary ni-

trogen excretion previously described (1) had passed its peak and was declining, and urinary creatine excretion was also beginning to recede from high levels. Urinary calcium excretion was approaching a peak, and, from the data of other patients who were not given gonadal steroids, would be expected to maintain a high plateau level for many weeks (2). Testosterone propionate was given to two male patients, D.A. and R.W., beginning at the 27th and 28th days after onset of illness, respectively (Figures 1 and 2). Each received the steroid for 30 days, D.A., 25 mgm. daily for the first 18 days and then 50 mgm. daily, and R.W., 25 mgm. daily throughout.

During the month's administration of t.p. to these two patients, urinary nitrogen excretion steadily decreased (from 20.0 gm. per day to 13.8 gm. per day in D.A. and from 18.4 gm. per day to 12.9 gm. per day in R.W.), and the nitrogen balance approached equilibrium. The rate of decline, however, was not greater than that occurring during the week just prior to the administration of t.p. Evidence for an effect of the hormones appeared in a rebound rise in urinary nitrogen (to 18.8 gm. per day in D.A. and to 16.6 gm. per day in R.W.) during the 12 days immediately following hormone administration. Urinary creatine excretion during t.p. administration did not fall but fluctuated about initial levels (1.00 gm. per day in D.A. and 0.725 gm. per day in R.W.); other patients not on t.p. showed a gradual decline in creatinuria during this phase of the disease. When testosterone was stopped, a distinct increase in creatinuria was noted. Urinary glycocyamine, the

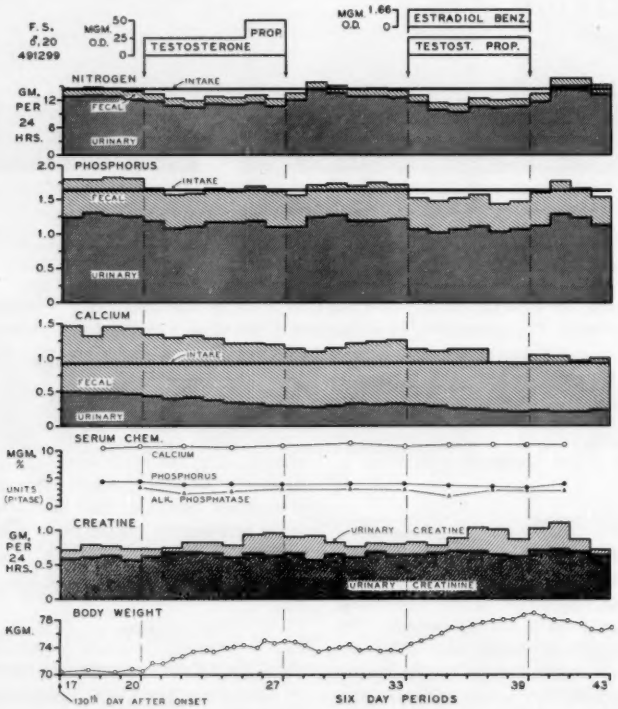

FIG. 3. METABOLIC DATA ON PATIENT F.S., 20-YEAR-OLD MALE, DURING THE FIFTH THROUGH TENTH MONTHS AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE AND COMBINED STEROID GIVEN DURING THE LATE CONVALESCENT-CHRONIC PHASE

Explanation of construction of chart is in legend for Figure 1. As indicated, urinary creatine in this patient exceeded urinary creatinine.

known precursor of creatine, was determined in an effort to obtain information suggestive of changes in creatine synthesis (1); the excretion values of this substance were not significantly altered. Urinary creatinine continued to fall slowly, at a rate not evidently different from that of other patients not on the hormone. Body weight, previously declining rapidly, remained fairly stable during the 30-day testosterone administration interval, then fell again rather rapidly when the hormone was stopped.

Urinary calcium excretion showed a definite decrease on testosterone, falling from 0.392 gm. per day to 0.300 gm. per day in D.A. and from 0.648 gm. per day to 0.443 gm. per day in R.W.; fecal calcium was slightly diminished. Calcium balance became less negative by nearly 0.200 gm. per day in each patient. As was usually observed in these studies, the decrease in calcium excretion continued for two metabolic balance periods (twelve days) after the hormone was discontinued. Thereafter, increases in urinary calcium were sluggish and slight so that both urinary calcium and calcium balance tended to remain at a new level close to that reached at the conclusion of hormone administration. Phosphorus excretion changed closely with nitrogen. Urinary citric acid was measured because of its importance for the solubility of calcium (2, 19); in the case of D.A., citric acid varied little from the control level of 0.364 gm. per day, but in R.W. it fell from 0.381 gm. per day to an average of 0.209 gm. per day during t.p. Except for a fall of 0.5 mgm. per cent in serum phosphorus in R.W., no changes occurred in the serum chemistries. Urinary 17-ketosteroids increased from 9.1 to 28.0 mgm. per day in D.A. but only from 11.5 to 15.9 mgm. per day in R.W. The increase in dosage from 25 mgm. to 50 mgm. of t.p. daily in D.A. brought about no detectable modification in any of the changes in metabolic function described.

b. Convalescent phase. Testosterone propionate was given to two male patients, F.S. and C.R., five months after onset of their disease. Each was given 25 mgm. daily for 30 days, then 50 mgm. for the next 12 days (Figures 3 and 4). In these two individuals in whom two-thirds or more of their original total muscle mass was without innervation, excretion of most metabolic constituents was fairly stable by this time; nitrogen was

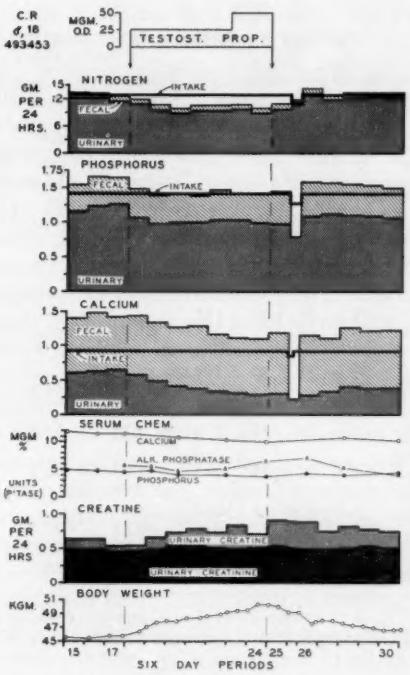

FIG. 4. METABOLIC DATA ON PATIENT C.R., 18-YEAR-OLD MALE, DURING THE FIFTH THROUGH EIGHTH MONTH AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE GIVEN DURING THE CONVALESCENT PHASE

Stools were not collected for a three-day interval between metabolic periods 25 and 26; patient had an intercurrent aspiration pneumonitis (see case summary). Explanation of construction of chart is in legend for Figure 1. As indicated, urinary creatine in this patient exceeded urinary creatinine.

virtually in balance, creatinuria was constant (approximately at 0.750 gm. per day for F.S. and 0.600 gm. per day for C.R.), urinary calcium excretion was at a steady high level (0.480 gm. per day for F.S. and 0.650 gm. per day for C.R.) and calcium balance was negative to the extent of approximately 0.500 gm. per day in each patient.

Testosterone propionate brought about a marked retention of nitrogen. When retention became maximal during the third week of hormone administration, daily positive nitrogen balance was 2.57 gm. for F.S. and 2.70 gm. for C.R.; the average daily storage throughout with respect to the control base-line (comparing the daily balances of control and experimental periods) was 1.68 and

TABLE I

Summary of principal metabolic changes resulting from administration of testosterone propionate and estradiol benzoate given singly to five patients *

Patient	Steroid and daily dose	No. of days	Initial day	N bal. change (Control to av. on steroid)	Total N storage	Rebound loss, % of storage	Creatinuria change (Control to max.)	Glycocyamine change	Urine calcium change (Control to maximum†)		Calcium balance change (Control to max.†)	Calcium balance change (Control to av.†)
	mgm.			*gm./day*	*gm.*		*gm./day*	%	*gm./day*	%	*gm./day*	*gm./day*
D. A.	T.p., 25	18	27									
	T.p., 50	12		+4.34			+0.007	0	−0.092	−23	+0.180	+0.065
R. W.	T.p., 25	30	28	+3.52			+0.131	0	−0.205	−32	+0.195	+0.061
F. S.	T.p., 25	30	155									
	T.p., 50	12		+1.68	70.6	13	+0.200		−0.202	−43	+0.325	+0.223
C. R.	T.p., 25	30	158									
	T.p., 50	12		+2.33	98.0	8	+0.241		−0.354	−56	+0.352	+0.210
R. W.	T.p., 25	24	232	+2.24	53.8	4	+0.178	+25	−0.035	−19	+0.063	+0.038
R. V. K.	T.p., 50	18	615	+2.35	42.3	7	+0.154		−0.030	−23	+0.092	+0.092
D. A.	E.b., 1.66	18	219						+0.061	+41		
	E.b., 3.32	12		−0.68	−20.4		+0.349	+90	−0.005	− 3	+0.033	−0.002
R. W.	E.b., 1.66	18	190	−0.88	−15.8		+0.178	+72	−0.080	−33	+0.079	+0.079

* Total N storage and rebound loss not calculated where control base-line was changing rapidly.
† Including first two metabolic periods (12 days) after steroid discontinued.

2.33 gm., respectively. Total nitrogen storage for the six weeks was 70.6 gm. for F.S. and 98 gm. for C.R., equivalent to 5 lbs. of muscle protoplasm for F.S. and 7 lbs. for C.R. Only approximately 10 per cent of this nitrogen storage was lost during the rebound phase after testosterone was stopped.

Creatinuria remained unchanged on t.p. for 10 to 15 days, then gradually and progressively increased by approximately 0.200 gm. per day. When t.p. was discontinued, creatinuria in F.S. remained at the same elevated level for approximately 12 days and then subsided to its original value; in C.R. urinary creatine for approximately 12 days rose an additional 0.070 gm. per day, then fell back toward the control level. The glycocyamine method had not been set up at this stage of the investigation so that analyses for this constituent were not carried out in these two patients. Urinary creatinine appeared to be increased slightly in both patients, the rise amounting to 0.050 gm. per day. Body weight increased 4.3 and 4.5 Kg. in F.S. and C.R., respectively.

Urinary calcium declined on t.p. and was reduced from 0.475 gm. per day to 0.273 in F.S. and from 0.637 gm. per day to 0.283 in C.R. by the second metabolic balance period after hormone administration. Fecal calcium was virtually unchanged so that the shifts of calcium balance toward positivity were equal to the decreases in urinary calcium. As described with D.A. and R.W., there was a carry-over of the effect of t.p. on calcium for approximately twelve days after discontinuation of the hormone; thereafter, urinary calcium rose slightly and calcium balance became more negative. Urinary citric acid decreased 0.270 gm. per day in F.S. and 0.359 in C.R. A fall of 1.1 mgm. per cent occurred in C.R.'s serum phosphorus and possibly a slight rise in his alkaline phosphatase. Urinary 17-ketosteroids rose from 10.0 mgm. per day to 23.6 in F.S. and from 6.3 to 17.2 in C.R.

Increasing the dose of testosterone from 25 to 50 mgm. daily appeared to have some influence although it was difficult to be certain since the higher dosage was given for only twelve days. Urinary nitrogen and citric acid seemed to be further depressed and urinary creatine and 17-ketosteroids further elevated. No change could

be detected in the trend of progressive decrease in calcium excretion.

c. Chronic phase. More than six months after onset testosterone propionate was given to four patients. R.W. was given 25 mgm. daily for 24 days and R.V.K. (a female) 50 mgm. daily for 18 days. Less information was obtained from the administration of 50 mgm. daily for 12 days to J.W. (a female) and 25 mgm. daily for the same interval to C.R., the latter observation without a control period following. In this phase, prior to hormone administration, excretion of all metabolic constituents was virtually unvarying. Nitrogen balance was slightly positive, and there was a persistent spontaneous creatinuria. Calcium balance was still negative in three of the four patients, in R.W. − 0.075 gm. per day at the 8th month after onset, in C.R. − 0.291 gm. per day at the 10th month and in J.W. − 0.093 gm. per day at the 11th month; R.V.K. at the 21st month was in positive calcium balance, + 0.147 gm. per day.

The following data obtained in R.W. and R.V.K. (Figures 2 and 5), who were given t.p. for longer periods than the other two patients, best describe the results obtained during the chronic phase. Pronounced nitrogen retention was noted in both patients, the average daily storage with respect to control base-line being 2.24 gm. for R.W. and 2.35 gm. for R.V.K. R.W.'s rebound loss of nitrogen after cessation of the hormone was only 4 per cent and R.V.K.'s 7 per cent. In both patients there was a progressive increase in urinary creatine of more than 0.150 gm. per day when t.p. was given and a brief further increase when it was stopped, the after-increase being more marked in R.W. whose paralysis was less extensive. A definite increase in urinary glycocyamine occurred in R.W. (0.110 gm. per day to 0.138), but no analysis of the constituent was made in R.V.K. Urinary creatinine barely changed in R.V.K. but increased approximately 0.100 gm. per day in R.W.

Calcium excretion was not greatly affected by these rather short exposures to the administration of testosterone. The positive calcium balance in R.V.K. was raised by 0.092 gm. per day, but in R.W. the reduction in negative balance was only 0.063 gm. per day, and in both patients urinary calcium was reduced by only 0.030 gm. per day. Urinary citric acid was reduced by only 0.074 gm. per day in R.W., but in R.V.K. citric acid excretion fell from 0.776 to 0.430 gm. per day. R.W.'s serum phosphorus declined 0.6 mgm. per cent. Urinary 17-ketosteroids increased from 15.4 to 18.9 mgm. per day in R.W. and from 8.4 to 27.2 mgm. per day in R.V.K.

In the two patients, C.R. and J.W., given t.p. for only 12 days, similar changes were found: an increase in positivity of nitrogen balance, a decrease in urinary calcium and citric acid, and a decrease in negativity of calcium balance. In C.R. a slight decrease in creatinuria was noted whereas in J.W. the more usual finding in these studies was noted, an increase in creatinuria on t.p., then a further rise following.

In all of the testosterone studies urinary phosphorus and phosphorus balance moved with the changes in nitrogen and calcium. Measured phosphorus balances agreed closely with theoretical phosphorus balances calculated on the basis of the relationship of these two elements to phosphorus in muscle and bone (20). Fecal phosphorus decreased less than 0.100 gm. per day in all instances. One other finding, quite consistent in all of the testosterone studies, was an increase in the 24-hour average urinary pH of approximately 0.2 of a pH unit.

2. *Effects of alpha-estradiol benzoate administration.* During the late convalescent-chronic phase estradiol benzoate was given to two male patients, D.A. and R.W. D.A. received 1.66 mgm. daily for 18 days and then 3.32 mgm. for the following 12 days. R.W. received 1.66 mgm. for 18 days. At this time (sixth and seventh months after onset of illness) nitrogen balance was slightly positive and creatinuria was fairly constant (0.464 gm. per day for R.W. and 0.666 gm. per day for D.A.). Calcium balance was − 0.109 gm. per day in R.W. and + 0.011 gm. per day in D.A.

In both patients, while on e.b. and over the first 12 days following, urinary nitrogen excretion was *increased* by approximately 1 gm. daily so that nitrogen balance became slightly negative. Urinary creatine promptly began to rise, the increase amounting to 0.178 gm. per day in R.W. and 0.349 gm. per day in D.A. at the time the hormone was discontinued; thereafter creatinuria did not rise further but began to subside within a few days. Glycocyamine excretion increased from 0.088 to 0.151 gm. per day in R.W. and from 0.103 to 0.196

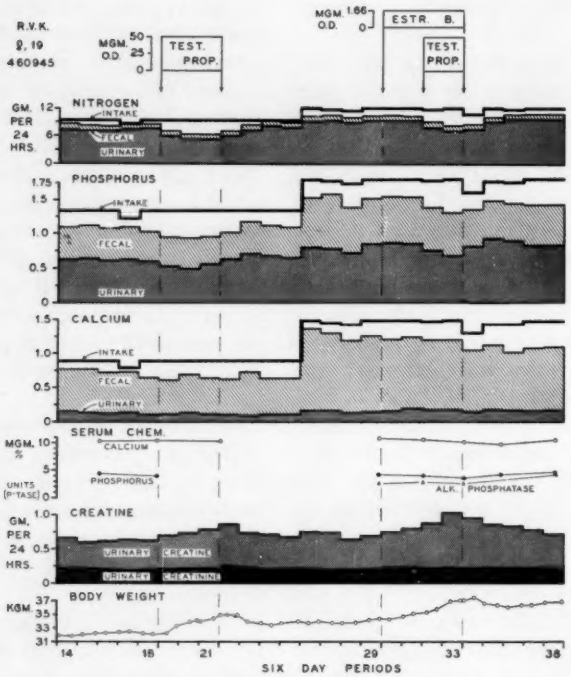

FIG. 5. METABOLIC DATA ON PATIENT R.V.K., 19-YEAR-OLD FEMALE, DURING THE 20TH THROUGH 24TH MONTH AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE AND COMBINED STEROID GIVEN DURING THE CHRONIC PHASE

Explanation of construction of chart is in legend for Figure 1. As indicated, urinary creatine in this patient exceeded urinary creatinine.

gm. per day in D.A. The latter showed a brief, unsustained rise in urinary creatinine the second week on e.b., while in R.W. no change in creatinine occurred.

Results with respect to calcium were quite different in the two patients. R.W. showed a sharp drop in urinary calcium which reached a level 0.100 gm. per day below control (average of periods on estradiol, 0.060 gm. per day less than control) and also showed a slight decrease in fecal calcium so that the reduction in negative balance was 0.079 gm. per day. Urinary calcium in D.A. showed a *rise* of 0.061 gm. per day on the 1.66-mgm. dose, then fell toward the control level when the dose was raised. Fecal calcium was decreased, however, so that no net change occurred in calcium balance. Urinary citric acid excretion more than doubled in both patients. No changes of note occurred in the serum chemistries. Urinary 17-ketosteroid excretion decreased slightly in both patients, from an average control value of 11.9 mgm. per day to 9.3 in R.W. and from 11.0 mgm. per day to 7.3 in D.A.

In both studies measured phosphorus balances agreed closely with theoretical phosphorus balances calculated on the basis of the relationship of nitrogen and calcium to phosphorus in muscle and bone. No change occurred in the 24-hour average urinary pH.

Assessment of the data of D.A. for the relative effects of the two dosage levels of steroid was difficult because in a number of instances increased changes occurring on the higher dose might have been due merely to a cumulative effect with time.

The added increases in urinary nitrogen and creatine, however, did seem related to higher dosage since the excretion of these substances had become relatively stable before the amount of e.b. was increased.

3. *Effects of combined androgen and estrogen administration.*

a. Convalescent phase. Effects of combined hormone administration were observed in three patients during the convalescent phase. R.W. (Figure 2) during the 4th month after onset of illness was given 25 mgm. of testosterone propionate and 1.66 mgm. of estradiol benzoate daily for 18 days. During the same month D.A. (Figure 1) was given the hormones in the same dosage for 18 days, then for the following six days 3.32 mgm. estradiol benzoate plus 25 mgm. testosterone propionate. Both R.W. and D.A. had shortly before begun to sit in a wheelchair for a few hours daily, but they were still in negative nitrogen and calcium balance. The studies on J.C. are described later.

On the two hormones urinary nitrogen excretion fell and nitrogen balance shifted from − 0.48 gm. per day for both R.W. and D.A. to an average of + 2.07 gm. per day for R.W. and + 2.42 for D.A., the average daily storage with respect to control base-line being 2.55 and 2.80 gm. per day, respectively. Rebound loss, however, was 37 per cent and 27 per cent, respectively, of the total amounts stored. Creatinuria rose promptly and progressively in R.W., from 0.490 gm. per day to 0.608, but in D.A. decreased by 0.148 gm. per day during the first 18 days, then rose above the control level coincident with increase in the dose of e.b. In both patients creatinuria rose even higher when the hormones were discontinued. Urinary glycocyamine excretion was increased approximately 50 per cent in both patients, from 0.083 to 0.134 gm. per day in R.W. and from 0.094 to 0.152 gm. per day in D.A. Urinary creatinine rose slightly; in D.A. this occurred after the hormones were stopped.

Urinary calcium decreased substantially in both patients, in R.W. from 0.429 gm. per day to a low of 0.226 gm. per day during the second metabolic balance period after the hormones were stopped and in D.A. from 0.298 gm. per day to 0.181, also during the second period following. Fecal calcium was reduced in both patients, but it is doubt-

TABLE II

Summary of principal metabolic changes resulting from administration of testosterone propionate and estradiol benzoate combined given to five patients *

Patient	Steroid and daily dose	No. of days	Initial day	N bal. change (Control to av. on steroid)	Total N storage	Rebound loss, *% of storage*	Creatinuria change (Control to max.)	Glycocyamine change	Urine calcium change (Control to maximum†)		Calcium balance change (Control to max.†)	Calcium balance change (Control to av.†)
	mgm.			*gm./day*	*gm.*		*gm./day*	*%*	*gm./day*	*%*	*gm./day*	*gm./day*
D. A.	T.p., 25 E.b., 1.66‡	24	99	+2.89	69.4	27	−0.148 +0.027	+62	−0.117	−39	+0.162	+0.057
R. W.	T.p., 25 E.b., 1.66	18	106	+2.55	45.9	37	+0.118	+61	−0.203	−47	+0.274	+0.208
J. C.	T.p., 25	18	70	+3.67			−0.325	+31				
	T.p., 25 E.b., 1.66	18		+4.46			−0.110	+63	−0.195	−39	+0.215	+0.143
J. C.	T.p., 25	18	190	+1.57	28.3		−0.105	+15				
	T.p., 25 E.b., 1.66	18		+1.74	31.3 59.6	61	+0.145	+35	−0.101	−44	+0.175	+0.120
F. S.	T.p., 25 E.b., 1.66	36	233	+2.43	87.5	29	+0.247	+80	−0.116	−36	+0.313	+0.184
R. V. K.	E.b., 1.66	12	681	+0.02					+0.057			
	E.b., 1.66 T.p., 50	12		+1.76	21.4	0	+0.367		+0.040	+28	+0.047	+0.037

* Total N storage and rebound loss not calculated where control base-line was changing rapidly.
† Including first two metabolic periods (12 days) after steroid discontinued.
‡ E.b., 3.32 mgm. daily given during final 6-day period.

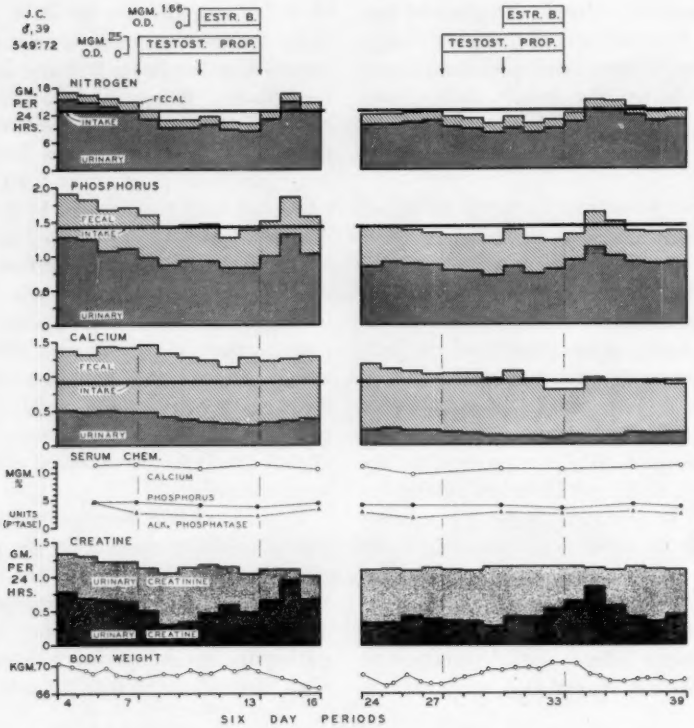

FIG. 6. METABOLIC DATA ON PATIENT J.C., 39-YEAR-OLD MALE, DURING SECOND TO FOURTH MONTH AND SIXTH TO NINTH MONTH AFTER ONSET OF PARALYTIC ACUTE ANTERIOR POLIOMYELITIS, SHOWING THE EFFECTS OF TESTOSTERONE PROPIONATE AND ADDED ESTRADIOL BENZOATE GIVEN DURING THE CONVALESCENT AND LATE CONVALESCENT-CHRONIC PHASES

Explanation of construction of chart is in legend for Figure 1.

ful that the degree of reduction was significant. The reduction in negative calcium balance in R.W. was 0.274 gm. per day, and in D.A. calcium balance changed from − 0.094 to + 0.068 gm. per day, a shift of 0.162 gm. per day. Urinary citric acid rose less than 0.100 gm. per day while the hormones were being given, but when they were discontinued, citric acid rose briefly an additional 0.250 and 0.170 gm. per day, respectively. Both patients showed a fall in serum phosphorus, in R.W. amounting to 0.7 and in D.A. 0.9 mgm. per cent. Urinary 17-ketosteroids increased from a mean control value of 10.3 mgm. per day in R.W. to 18.8 and in D.A. from 9.9. mgm. per day to 16.8.

J.C. (a male) on two occasions was given testosterone propionate 25 mgm. daily for 36 days, for the last 18 days of which he was also given 1.66 mgm. daily of estradiol benzoate (Figure 6). The first course was given during the third and fourth months and the second during the seventh and eighth months.

At the time the hormones were first given to J.C., nitrogen, calcium and phosphorus balances were all negative, creatinuria was at a high level and urinary creatinine was still gradually falling. During the first 18 days, on t.p. alone, nitrogen balance shifted from an average of − 2.48 gm. per day to an average of + 1.19 gm. per day; when e.b. was added, there was a further storage of nitrogen, the average balance being + 1.98 gm. per day. The total accumulated positive nitrogen balance was 57 grams; if calculated relative to the change from control base-line, storage would

be considerably greater but would probably not be accurate because of the fact that the base-line was shifting. Rebound loss of nitrogen after cessation of the hormones was also difficult to calculate for the same reason; on the basis of absolute balance rebound loss was 61 per cent. In contrast to the other patients, J.C. showed a decrease in creatinuria on testosterone, from 0.655 gm. per day to 0.330; when estradiol was given, creatinuria increased again nearly to control levels. When the hormones were discontinued, creatinuria rebounded sharply to a level 0.300 gm. per day above control. Glycocyamine excretion began to increase on t.p., rose further on e.b. and t.p. combined and returned gradually to control levels 30 days after the two hormones were stopped; the control level was 0.122 gm. per day and the final peak on steroids 0.199 gm. per day. The effect of the hormones on urinary creatinine was apparently to bring about a temporary halt in the progressive fall to that point.

From examination of the decreases in urinary calcium excretion and negative calcium balance, it is difficult to determine whether estradiol added to the effect of testosterone; the total change in the 36-day interval was a fall of 0.195 gm. per day in urinary calcium and 0.215 gm. per day in the negative balance. Urinary citric acid excretion on t.p. *increased* in this patient from 0.636 gm. per day to 0.770, and continued to rise an additional 0.100 gm. per day when e.b. was added; citric acid did not rise further when steroid administration was interrupted. Serum phosphorus progressively decreased, the total decline being 0.6 mgm. per cent. Urinary 17-ketosteroids rose from 6.6 mgm. per day to 11.8 during the 18 days on t.p., then remained at the same level while e.b. was also given.

When androgen and estrogen were administered again in the same fashion beginning during the seventh month, the resultant changes differed principally in degree and were, in general, slightly less marked. Nitrogen balance was already slightly positive, became more positive on t.p. to the extent of 1.57 gm. per day on the average, then nitrogen balance remained virtually the same with e.b. added. Total nitrogen storage with respect to the control balance was 60 gm., and the rebound loss afterward, 61 per cent; these figures are similar to those stated for the first course of the hormones in this patient but were calculated on a different basis. The pattern of change in creatinuria was similar to that during the first course of these steroids, the chief differences being that the levels rose to a greater extent when estrogen was added and during the rebound following. From a control level of 0.384 gm. per day, creatinuria dropped to a 0.279 gm. per day average during the third metabolic balance period, then rose to 0.529 gm. per day in the sixth; during the second period following, the level was 0.847 gm. per day. The changes after the third period on steroid were probably not due specifically to estrogen since a similar pattern of fall and then rise occurred in D.A. who was given e.b. with t.p. from the start. As in the first course of the two steroids in J.C., urinary glycocyamine was increased, from 0.174 gm. per day to 0.234. Urinary creatinine rose very slightly.

Decline in urinary calcium was much less than during the first course, 0.101 gm. per day, but the shift in calcium balance, − 0.140 to + 0.035 gm. per day, was nearly the same. Changes in urinary citric acid differed from those of the first course; from a high control level of 0.871 gm. per day, on t.p. alone a definite fall occurred (to 0.669 gm. per day) which was only slightly reversed on e.b. (to 0.738 gm. per day). On cessation of the hormones citric acid rose over the first twelve days to an average of 0.828 gm. per day. A fall in serum phosphorus was again noted (0.8 mgm. per cent). Urine was not analyzed for 17-ketosteroids during this phase of the study.

b. Chronic phase. The effects of combined androgen and estrogen during the chronic phase were tested in two patients. The principal observation was made on F.S. (a male), who eight months after onset was given daily for 36 days 25 mgm. of testosterone propionate and 1.66 mgm. of estradiol benzoate. In a shorter study, R.V.K. (a female) 22 months after onset was given estradiol benzoate 1.66 mgm. daily for 24 days and during the last 12 days was also given 50 mgm. of testosterone propionate daily.

In the study on F.S., peak nitrogen retention was + 3.35 gm. per day during the third six-day metabolic balance period; balance shifted from + 0.06 gm. per day during the preceding control balance periods to an average of + 2.49 over the 36 days on the hormones. Total nitrogen storage was 89.6 gm.; the rebound loss over the following

24 days was 29 per cent of the amount stored on the hormones. Urinary creatine changed little over the first 12 days on steroids, then rose to a peak during the fourth period which was 0.247 gm. per day above the control level; after steroid administration creatinuria increased an additional 0.070 gm. per day. Urinary glycocyamine increased steadily from a control level of 0.126 gm. per day to a peak of 0.240 gm. per day during the fifth period; glycocyamine returned to the control level 21 days after androgen-estrogen had been stopped. Urinary creatinine showed some irregular elevations which were of doubtful significance.

Urinary calcium in F.S. decreased from 0.319 gm. per day to 0.203 gm. per day by the second period after hormone administration. Fecal calcium appeared to be slightly but definitely reduced, and the calcium balance shifted from − 0.300 gm. per day to + 0.013. Urinary citric acid rose from 0.747 gm. per day to 0.900 gm. per day without an appreciable rise after the hormones were stopped. The usual decline in serum phosphorus was observed, amounting in this case to 0.7 mgm. per cent. Urinary 17-ketosteroids rose slightly on the steroids from 14.4 to 17.4 mgm. per day, but fell to 7.9 mgm. per day three weeks following.

Administration of the hormones to R.V.K. was brief, but it did afford an opportunity to observe initially the effect of estrogen followed by the addition of androgen. Nitrogen balance was unchanged by e.b. but shifted from + 1.55 gm. per day to an average of + 3.29 gm. per day when testosterone was added; administration of the hormones was so brief that total nitrogen storage and rebound loss were both extremely small. Creatinuria increased promptly and rose from 0.662 gm. per day to a peak of 1.029 gm. per day in the final combined period. After the hormone administration, creatinuria did not continue to rise but fell gradually over the next 24 days. The urine was not analyzed for glycocyamine. Creatinine was increased by 0.030 gm. per day, a 15 per cent change in this individual. As occured with one other patient (D.A.) on estrogen alone, urinary calcium *increased* from 0.141 gm. per day to 0.198 during the second balance period and remained elevated when t.p. was also given. The improvement in calcium balance was not significant. Urinary citric acid rose on e.b. (0.448 gm. per day to 0.671) and was promptly depressed back to the control level when t.p. was added. The fall in serum phosphorus amounted to 0.5 mgm. per cent. Urinary 17-ketosteroids from the second period on e.b. to the final combined period rose from 8.6 to 19.9 mgm. per day.

In all of the studies involving combined hormone administration, urinary phosphorus shifted with nitrogen and calcium. Measured phosphorus balances agreed closely with theoretical phosphorus balances calculated on the basis of the relationship of phosphorus to nitrogen and calcium in muscle and bone, respectively. Fecal phosphorus was reduced by 0.120 gm. per day in the study of F.S., but in all other studies of combined steroids changes in fecal phosphorus were minor, being less than 0.080 gm. per day.

B. *Changes in electrolyte excretion and balance*

Table III summarizes the data with respect to effect of the steroids on sodium and potassium balances and on urinary chloride excretion. Behavior of these elements was somewhat variable from one patient to another, but from a review of the data of the group a general pattern emerged. Sodium tended to be stored fairly promptly upon administration of the hormones, and in a few instances *marked* retention occurred during the first metabolic balance period, as indicated in the table. With regard to average sodium balance over the entire interval of steroid administration, however, a difference appeared in the effect on sodium between testosterone alone and when combined with estradiol, the combination causing sustained sodium retention while testosterone alone did not. This difference becomes evident if the testosterone data of D.A. and R.W. (I)[3] are excluded. In these two studies t.p. was given relatively soon after onset of illness while hot packs were still being applied; the sodium excretion through the sweat, therefore, was undoubtedly considerable and variable so that sodium balances in these two studies were inaccurate. In the remaining three studies of t.p. alone mean sodium balances on the hormone were virtually identical with the mean control sodium balances. Nevertheless, on cessation of t.p. all three patients had a sodium diuresis.

[3] (I) following patient's initials indicates the first of two courses of similar hormone administration, (II) the second.

TABLE III

Summary of data on potassium, sodium and water balance and urinary chloride excretion, effects of testosterone propionate and estradiol benzoate given singly and combined

Patient	Steroid	Initial day	Potassium balance			Sodium balance				Urinary chloride			Water balance		
			Control	Av. on steroid	Post-control†	Control	First period	Av. on steroid	Post-control	Control	Av. on steroid	Post-control	Control	Av. on steroid	Post-control
			gm./day	gm./day	gm./day	gm./day	gm./day	gm./day	gm./day	gm./day	gm./day	gm./day	ml./day	ml./day	ml./day
D. A.	T.p.	27*	−0.037	+0.212	−0.360	+0.029	+0.158	+0.449	−0.022	5.66	5.58	5.80	+903	+771	+298
R. W. (I)	T.p.	28*	+0.089	+0.080	−0.115	+0.091	−0.030	+0.508	+0.212	5.41	5.44	5.67	+593	+380	+272
F. S.	T.p.	155	+0.359	+0.586	+0.156	+0.180	+0.048	+0.045	−0.157	6.04	5.95	6.24	+151	+259	−101
C. R.	T.p.	158	+0.246	+0.409	+0.197	+0.314	+0.510	+0.322	−0.136	5.63	5.34	5.84	+330	+481	+171
R. W. (II)	T.p.	232	+0.518	+0.418	+0.344	+0.620	+0.697	+0.738	+0.192	5.30	5.06	5.40	+547	+695	+913
R. V. K.	T.p.	615	−0.219	+0.340	−0.112										
Mean of F. S., C. R., R. W. (II)			+0.374	+0.471	+0.232	+0.371	+0.418	+0.368	−0.034	5.66	5.45	5.83	+343	+478	+328
D. A.	E.b.	219	+0.264	+0.087	+0.074	−0.185	+0.761	+0.387	−0.229	5.24	5.36	5.96	+491	+365	+196
R. W.	E.b.	190	+0.092	+0.060	−0.040	+0.458	+0.446	+0.502	+0.491	5.30	4.84	5.27	+605	+569	+388
D. A.	Comb.	99	+0.310	+0.642	+0.545	+0.361	+0.787	+0.518	−0.102	5.62	5.24	6.11	+339	+651	+234
R. W.	Comb.	106	+0.075	+0.452	−0.086	+0.254	+1.068	+0.855	+0.180	5.56	4.73	5.57	+530	+569	+548
J. C.	Comb.	70	−0.232	+0.427	−0.319								+821	+849	+485
J. C.	Comb.	190	+0.235	+0.468	−0.175								+732	+951	+594
F. S.	Comb.	233	+0.277	+0.704	+0.677	+0.262	+0.271	+0.439	+0.387	5.84	5.58	5.80	+134	+175	+256
R. V. K.	Comb.	681	+0.176	+0.018	−0.107								+ 82	+110	+ 7
Mean	Comb.		+0.140	+0.452	+0.089	+0.292	+0.709	+0.604	+0.155	5.67	5.18	5.83	+440	+551	+354

* Hot pack therapy.
† Post-control = First 12 days after steroid discontinued.

On combined steroids, however, sodium balance was distinctly more positive (by 0.312 gm. per day on the average) than during the control periods; on stopping the hormones, balance was less positive than during control in two of the three studies. On e.b. alone one patient, D.A., showed definite sodium retention whereas the other patient, R.W., showed virtually no change in sodium balance.

Urinary chloride excretion was only slightly decreased (less than 4 per cent) during t.p. administration and slightly increased afterward. On combined hormone, however, the decrease in urinary chloride was more definite (9 per cent); again, there was a slight increase in chloride excretion following. On e.b. one patient showed a decrease similar in magnitude to that noted on combined steroid and one patient showed no change.

Crude water balances, based on fluid intake and output only, also showed a considerable degree of variation in certain patients. These data do not include calculations for the water of combustion of food or any estimates of changes in insensible water loss. Periodic determinations of the basal metabolic rate suggested that insensible water loss was probably increased during the early weeks. During this period water was probably also being lost in considerable amounts through the skin due to hot packs. These routes of probable additional water loss might account for the apparently large positive water balances during the control phases prior to t.p. administration in D.A. and R.W. (I). Review of data in the other studies indicated a consistent tendency to retain small amounts of fluid on t.p. and on combined steroid as compared with the control water balances and a variable tendency to return to the control balance level after hormone was stopped. In five studies the data suggested a water diuresis upon discontinuation of the steroids, the water balance during the first two balance periods following being more than 300 ml. per day less positive than during steroid administration; in two patients the balance became more positive during the first two periods following steroid. In the two estrogen studies crude water balances were less positive on the steroid and again less positive thereafter.

Potassium balances indicated that this element tended to be stored or lost as nitrogen was stored or lost. On testosterone alone and on combined t.p. and e.b., potassium balances were more positive than during control periods, and when the hormones were stopped, potassium balances were less positive coincident with the rebound loss of nitrogen. Exception was noted in both the early and late t.p. studies of R.W. in which potassium balance did not become more positive when nitro-

gen storage was substantially increased. In explanation, the rare possibility is suggested of coincidental marked error in the true potassium intake during these phases, the potassium content of foods being notoriously variable; occurrence of this discrepancy, however, twice in the same patient and not in the other patients seems to call for a different interpretation, one which we are unable to offer. The potassium balance of R.V.K. on combined steroid was influenced by the fact that t.p. was given and nitrogen storage increased during the last two metabolic periods only so that the predominant influence was that of e.b. In the two studies of e.b. alone, potassium balances became less positive on the estrogen as did also the nitrogen balances.

In view of the report of Bower, Morgan, and Chaney (21) of excessive potassium excretion during the early weeks in a group of poliomyelitis patients, nearly all of whom needed a respirator, a brief account may be made of potassium excretion during the first few weeks in the four patients studied during that phase. Urinary potassium was observed to rise to a peak level of 3.3 to 5.5 gm. per day at the 11th to 13th day. At the level of potassium intakes given, negative potassium balances were maximally of the order of 2.0 gm. daily; negative potassium balance lasted until three to four weeks after onset. In comparison with the extensive losses of nitrogen (1), these potassium losses were modest, as judged by theoretical potassium balances calculated from the ratio of potassium to nitrogen in muscle (20); actual potassium losses were considerably less than the theoretical. Even if actual potassium losses had been high, however, they would not have evidenced particular loss of nerve tissue since nerve is not as rich in potassium as muscle (22).

II. *Tests of Physiological Function*

A. *Basal metabolic rate*

Determinations of basal metabolic rate were made approximately every 10 days to 2 weeks so that an average of 20 determinations was made during each patient's several months' study.

Among the seven patients studied from within the first two months after onset, five showed a gradual decrease in basal metabolism (calories per M.^{2} per hr.) over the first two to six months. Based on comparison of groups of two or more determinations, this decrease ranged from 9 per cent in D.Q., occurring over the first two months only, to 32 per cent in R.W. from the first to the sixth month. Mean decrease for the five patients was 18 per cent; this was equivalent to a decline of approximately 17 percentage points in the clinical reporting system of per cent variation from Aub-DuBois standards. The other two patients, M.O'M. and J.C., showed fluctuations with no definite change.

Review of tests performed during administration of testosterone propionate revealed no clear change in the studies of D.A. and R.W. (I) where the steroid was given beginning at approximately one month after onset. In F.S., C.R., and R.W. (II) later on, basal metabolism was raised by testosterone 5.4, 3.7 and 4.0 cal. per M.^{2} per hr., respectively, or by an average of 13 per cent (approximately equivalent to an increase of 11 percentage points in relation to Aub-DuBois standards); when the hormone was discontinued, basal metabolism fell again to control levels. In R.V.K. only single tests were made before and during testosterone administration.

On combined testosterone and estrogen basal metabolism of D.A. and F.S. increased 2.7 and 4.9 cal. per M.^{2} per hr. or 8 and 15 per cent, respectively. In R.W. and R.V.K. too few tests were carried out. In both administrations of steroid to J.C., tests showed modest variations and no clear trend. On estrogen alone, tests in D.A. indicated a 13 per cent rise, whereas during the short period that estrogen was given to R.W. only one test was performed.

B. *Muscle strength*

The pattern of gradually increasing muscle strength from the second week for five or more months was described in the first paper of this series. Ergometer tests made at intervals of every one to two weeks showed increases during steroid administration in several patients, but in none did the tests suggest that these increases might be due to hormonal influence rather than just part of the natural course of improvement.

C. *Leg girth*

During the first two to five months leg girth, measured every four to eight days, gradually decreased as involved leg muscles underwent varying degrees of atrophy (1). Hormonal administration during these first several months did not prevent nor alter the rate of this process.

When testosterone propionate was given five months or more after onset to four patients, a mean increase in calf circumference resulted amounting to 1.0 cm. (approximately 3 per cent) and in thigh girth 1.2 cm. (also approximately 3 per cent); a decrease of similar proportions occured over the first two weeks after the hormone was discontinued. Similar increases occurred with combined steroid, the average increase in calf circumference being 0.7 cm. and in the thighs 1.0 cm. Although electrolyte and water changes on testosterone and combined t.p. and e.b. were not marked, it is not possible to be at all certain that the increases in leg girth occurring on these steroids reflected increases in muscle mass.

On estrogen alone, D.A. showed no change in girth and R.W. an increase of only 0.4 cm. in both calves and thighs, an insignificant amount.

D. *Skin temperature*

Measurements of skin temperature with the Hardy radiometer were made in two patients. In F.S. combined t.p. and e.b. brought about a rise in the temperature of the feet of 1.8° C. and of the legs of 0.6° C., accompanied by no significant change in total average skin temperature over the whole body. Testosterone alone in this patient and in C.R. resulted in no consistent changes in skin temperature in any area.

E. *Clinical effects*

Experience with use of gonadal hormones in these studies was reviewed for possible additions to our information regarding their clinical masculinizing or feminizing effects. Predominant findings were evidence of individual variation among patients and of neutralization of the side effects of one hormone by the high dosage of another hormone, which then exerted its own effects.

Among the male patients, predominant in this group, five were given testosterone propionate alone. One patient noted an increased frequency of erections and transiently developed acne with 400 to 450 mgm. and another acne after 600 mgm. The latter patient when given testosterone a second time noted some difficulty starting his urinary stream after approximately 450 mgm. but did not develop acne by the time the steroid was stopped after a total dose of 600 mgm. A third patient (F.S.) reported increased beard growth and an increase in sexual interest and erections when he had received 750 to 900 mgm. The other two patients were given 1050 and 1350 mgm. total without clinical effects.

The two male patients given estradiol alone both developed areolar tenderness and palpable tissue beneath the areolae; R.W. did so after 30 mgm. of estradiol benzoate and D.A. after 50 to 60 mgm. (180,000 and 300,000 to 360,000 rat units, respectively).

Combined androgen and estrogen were given to males on five occasions, in each instance at a ratio of 15 mgm. t.p. to 1 mgm. of e.b. R.W. noted areolar tenderness after 30 mgm. of estradiol (180,000 rat units), the same accumulated dosage at which this symptom was noted in this patient when e.b. was given alone. F.S. also noted areolar tenderness at this dosage, but the discomfort subsided as the hormones were continued; this patient had noted "masculinizing" symptoms at 750 to 900 mgm. of t.p. alone but on combined steroids noted no such effects by the time steroids were discontinued after 900 mgm. of t.p. had been given. J.C., given combined steroids twice, had areolar area tenderness after 15 mgm. of e.b. during one course and after approximately 25 mgm. during the other (90,000 and 150,000 rat units, respectively). D.A. had no feminizing discomfort after a total of 50 mgm. of e.b. with 600 mgm. of t.p. even though during the final metabolic balance period the androgen: estrogen ratio was only 7.5 : 1.

R.V.K., a female, developed huskiness of the voice and facial acne after 650 mgm. of testosterone propionate when given alone and after 600 mgm. when given with estradiol benzoate. The ratio of the combined steroids given this patient was 30 : 1 by weight, testosterone propionate to estradiol benzoate.

COMMENT

The studies reported in this paper have indicated a number of metabolic and physiological effects of gonadal steroids in adult patients with paralytic poliomyelitis. The effects in general were similar to those previously reported in other conditions, but certain exceptions were noted.

Effects on calcium metabolism

Among the metabolic effects, probably the most important was the action of testosterone propionate and combined t.p. and estradiol benzoate in decreasing calcium excretion (7, 23). Onset of this action was slow, requiring as long as one to two weeks. Then, for the length of time the hormones were given in these studies (up to 42 days), the change was gradually progressive and tended to extend for up to 12 days after the hormones had been discontinued. Thereafter, urinary calcium excretion and balance generally returned sluggishly toward preceding levels. In a few instances, frequently during later months of the convalescent phase or during the chronic phase, calcium excretion remained more or less at the final level reached just following hormone administration. The pattern of action suggested that these hormones would produce an even greater total effect if given continuously or in repeated long courses rather than in the relatively brief investigative trials employed in these studies.

Reductive action on calcium excretion was shown by all three forms of hormone administration, androgen, estrogen and the combination of the two, but not consistently in the case of estrogen. The two estrogen studies were each of unusual interest, one because in it calcium storage occurred coincident with an *increase* in nitrogen excretion, the other because urinary calcium increased so that no net calcium storage resulted.

A number of previous studies have shown the efficacy of these hormones in conditions in which demineralization is already established, such as postmenopausal and senile osteoporosis (6, 12, 13), Cushing's syndrome (8–11) and acromegaly (14). The present studies suggest their possible usefulness either in the prevention of or in the reduction of the severity of mineral loss *as it is occurring*, in this and in other paralytic disorders or in other states in which immobilization plays a prominent part. Another potential benefit lies in the fact that the reduction in urinary calcium would lessen the likelihood of calculus formation; the accompanying reduction in urinary phosphorus would be additionally helpful. If these quantitative effects were combined with close attention to adequate urine volumes and if hormone combinations were given which would maintain satisfactory levels of urinary citric acid (see below), a decrease in incidence of urinary tract stone formation in immobilizing states seems reasonable to be expected.

Nitrogen sparing action

The nitrogen or protein sparing action of testosterone, alone or in combination with estrogen, was clearly demonstrated during the convalescent and chronic phases in these studies, as it has been consistently in many other observations (4, 10, 23–26). A question of interest is whether the data gave any indication of the location of the deposited protein. Comparison of the concomitant changes in excretion of phosphorus and sulphur indicated that nitrogen was being stored with these elements in the proportion in which they are found in muscle tissue. The measured phosphorus balances were always within 5 per cent (as related to the intake level) of theoretical phosphorus balances calculated from the balances of nitrogen and calcium, anticipating that phosphorus would be excreted or stored with these elements in the proportion found in muscle and bone (20). Calculation of the ratios of urinary nitrogen to urinary sulphur, balance period by balance period, revealed them to lie generally within the usual or normal range of 14:1 to 16:1 (1), but occasionally values as high as 17.5:1 were obtained. A frequent tendency toward higher ratios appeared during administration of testosterone and immediately following, suggesting the possibility at those times of more tenacious storage of sulphur-rich compounds.

The nitrogen-phosphorus-sulphur relationships indicated that protein was likely being stored in tissues having the composition of muscle, but no way was evident of determining whether there might be any predilection of the stored protein for denervated muscle, intact muscle or other tissues of similar composition. Reassurance that the pro-

tein was being stored mainly in muscle rather than in other tissues is afforded by the finding of Kochakian, Robertson, and Bartlett (27) that in rats given testosterone propionate protein was deposited in preferential order in carcass, then seminal vesicles and prostate, liver and kidney. An experimental study bearing directly on the question of possible protection of denervated muscle by gonadal steroids is that of Gordan, Feinstein, and Ralston (28); in this study the right sciatic nerve was resected in two of three groups of rats. Testosterone propionate did not affect the atrophy, length-tension relationships or histology of the denervated anterior tibial muscles. The contralateral normal muscles in these animals, however, were approximately 5 per cent heavier than the muscles of untreated control rats. From these experiments considered in conjunction with our own studies, it seems reasonable to suppose that protein stored under the influence of testosterone was being deposited principally in intact, uninvolved muscle fibers. Leg girth measurements in our studies did not provide information which would aid in establishing this point, such as greater increase in girth of uninvolved versus involved limbs. In all patients involvement was nearly equal bilaterally. In the one patient, R.W., who had less leg muscle impairment than the others, increase in girth on testosterone was no greater, although his period on t.p. was somewhat shorter than that of the other patients (24 versus 42 days).

It is important to note that a definite protein-sparing action of testosterone was not obtained during the period of excessive nitrogen excretion in the first two months. In another situation embodying nitrogen loss, the period after injury, correction of negative nitrogen balance has been attempted by dietary means and found difficult (29).

The action of estrogen in these studies in *increasing* nitrogen excretion is contrary to the findings of Albright and Reifenstein in post-menopausal and senile osteoporosis (6, 12, 13) and of Knowlton, Kenyon, Sandiford, Lotwin, and Fricker (30) in normal women; these investigations had indicated a modest but definite nitrogen-sparing action. Reifenstein (25) summarized the results of studies using 1.66 mgm. of estradiol benzoate and found an average nitrogen retention of 16 mgm. per Kg. per day during days 5 to 15, equivalent to more than 1 gm. per day in an average sized individual at a daily intake of 13.5 gm. of nitrogen. In Cushing's syndrome, however, estrogen has been given to two cases (8) with similar findings to those of the present study in that an adverse effect on nitrogen balance developed accompanied by a slightly beneficial effect on calcium balance. Experimentally, a related effect is suggested by the production by stilbestrol of an increase in urinary glucose and nitrogen in normal and partially depancreatized rats (31).

The question of any influence of gonadal steroids upon fecal nitrogen excretion was reviewed by Kochakian (4) who reported that no effect was noted in seven studies of a considerable variety of patients. The present studies confirm these reports.

Creatine synthesis and storage

Testosterone propionate and methyl testosterone are generally regarded as having dissimilar effects on creatine metabolism. The 17-methyl compound causes an excess creatinuria and is believed to stimulate creatine synthesis and enhance creatine storage; the propionate is thought to favor only the storage of creatine (32). Kochakian (4) has stated that the effects of the two compounds may be alike and that there is only an apparent dissimilarity due to the likelihood that the 17-methyl group hastens these effects. Keutmann, Bassett, and Kochakian (33) measured the creatinuria after cessation of testosterone propionate in a patient with Cushing's syndrome, found it greater than the amount retained during administration of the hormone and concluded that the propionate increases both synthesis and storage of creatine. Our studies support this latter view. In one patient with more unaffected total muscle mass than the others, a decrease in creatinuria occurred indicative of increased storage. In the other patients, however, creatinuria gradually increased, suggestive of increased synthesis. In two instances (D.A. and R.W. [II]) the increase in creatinuria on testosterone was followed by a sharp additional increase reminiscent of the rebound rise seen in patients with normal muscle metabolism immediately following testosterone propionate; in these two instances evidence is visible of both increased synthesis and storage.

These various patterns may all be reconciled by the hypothesis that both synthesis and storage are aided by this steroid.

The indication that synthesis is increased by testosterone propionate is supported by the glycocyamine data. Urinary excretion of this substance was measured in three testosterone studies. In the second study of R.W. and in the two studies of J.C., the latter having been given testosterone alone for 18 days prior to combined steroid, substantial increases in urinary glycocyamine excretion occurred. The increased excretion of this known creatine precursor suggests that production of the glycine-amidine part of the creatine molecule had been increased in liver and kidney.

A different situation was apparently present with regard to the influence of testosterone on creatine synthesis during the first two months after onset, as indicated by absence of change of glycocyamine excretion in the two patients given testosterone during this early phase. This lack of response in glycocyamine occurred at a time when urinary creatine levels were still high so that the possibility comes to mind that an increase in creatine production by hormone may have been suppressed by the already copious amounts available and circulating. This situation may be similar to that noted in a study in progressive muscular dystrophy (34, 35) in which testosterone propionate failed to produce an increase in urinary glycocyamine; an increase in creatine storage occurred in these dystrophy patients since a rise in creatinuria resulted after the hormone was stopped.

In the two studies in which estradiol was given, a distinct increase was noted in creatinuria and in urinary glycocyamine indicative of a stimulation of creatine synthesis by this hormone. Since creatinuria began to fall within a few days after estradiol was stopped and since, in any event, there was no rebound rise in creatinuria similar to that following testosterone, it seems probable that little or no increase in creatine storage occurred under the influence of this estrogen. Increased urinary creatine excretion by an estrogen has been barely noted in previous studies. Knowlton, Kenyon, Sandiford, Lotwin, and Fricker (30) described an increase in creatinuria from 0.200 to 0.400 gm. per day on 5 mgm. of estradiol in a girl with adrenogenital syndrome but not on administration to a normal woman. From the charted data of Case 10 in the study of Williams, Whittenberger, Bissell, and Weinglass (36) of Addison's disease, there appeared to be a slight increase in creatinuria on 5 mgm. of stilbestrol given for six days. Because of the plan of the study of Deakins, Friedgood, and Ferrebee (37) of a patient with Cushing's syndrome, it was difficult to determine whether creatinuria had been influenced by stilbestrol. We have been unable to find measurements of urinary glycocyamine in previous studies of the effects of estrogens.

Increases in urinary creatinine in a number of instances during the course of testosterone and combined steroid administration reflected the suggested enhancement of creatine synthesis and storage. These increases in creatinine, however, occurred in only approximately one-half of the studies and quantitatively were usually small.

Changes in electrolyte excretion

Because of similarities in structure between the gonadal steroids and adreno-cortical hormones, Thorn and Harrop (38) suspected that androgenic and estrogenic preparations might have salt and water retaining properties. In normal male dogs they demonstrated (38, 39) reduction in urinary volume, sodium and chloride and a slight increase in urinary potassium by single injections of these steroids, estradiol and progesterone having the greatest sodium retaining effect and testosterone the least. In addition, estrone, alpha-estradiol and testosterone caused a decrease in urinary phosphate and nitrogen; testosterone propionate (25 mgm. daily) given to a normal male subject brought about a reduction in urinary potassium as well as in sodium, chloride, phosphorus and nitrogen (39). Thorn, Nelson, and Thorn (40) extended observations in dogs of the effect of ovarian hormones and testosterone on salt and water retention and investigated human pre-menstrual weight gain, finding it associated with sodium, chloride and water retention and the increased urinary excretion of "estrogenic substance."

The present study confirms Thorn's opinion that testosterone is a relatively weak sodium retainer in that sodium balances over several metabolic balance periods on this steroid averaged the same as the control balances; two of three patients,

however, showed distinct sodium retention during the first six days on testosterone and all showed sodium diuresis when it was withdrawn. Variation from patient to patient was emphasized by the marked sodium retention by one patient on estradiol benzoate and virtually none by the other patient. When the two steroids were given together, definite sodium retention consistently occurred, but here again individual variation was evident.

Urinary chloride excretion data were less variable than the sodium data, urinary chloride being diminished slightly in all three forms of hormone administration; no change occurred in one of the estradiol studies. Chloride diuresis after the hormones were discontinued occurred in five studies and did not in three.

The water balance data were consistent with respect to the effects of *testosterone* and *combined steroid* in that all studies with these two forms of hormone showed more positive water balances on steroid, by an average of a little more than 100 ml. daily. This is more than twice the amount of fluid storage associated with new protoplasm which would be anticipated (20) from the average nitrogen storage when testosterone or combined steroid were given (2.47 gm. N per day). Subsequent water diuresis occurred less consistently, although in five studies the water balance over the first twelve days after steroid was discontinued averaged more than 300 ml. per day less positive than during steroid administration.

Water balance data on the two patients on *estradiol,* however, recall the conflicting results obtained in various studies since those of Thorn. Knowlton and her co-workers (30) noted a decrease in urine volume on estradiol in one experiment and later noted no change in a second experiment on the same patient. Shapiro (41), who gave 7.5 mgm. of estradiol benzoate daily for five days to patients with secondary amenorrhea and to patients with diabetes insipidus, consistently obtained a decrease in urine volume followed by an increase when estradiol was stopped. On the other hand, Sharpey-Schafer and Schrire (42), who gave estradiol benzoate in approximately the same dosage as Shapiro for 7 to 10 days to 14 women, before and after the menopause, reported no decrease in urine volume. In our two males the latter result was largely obtained with perhaps a slight loss of water on estradiol and a further loss following. No evidence was found in our data for a transient storage of water during the first few days on estrogen as reported recently by Preedy and Aitken (43) using a larger dose.

Potassium balances became more positive on testosterone and combined steroid in association with nitrogen storage. On estradiol, potassium balances became less positive as did also the nitrogen balances. Although it is possible that these gonadal steroids may have a desoxycorticosterone-like action on the kidney, as has been suggested (39), apparently this action is obscured if the hormones provide sufficient stimulus to tissue growth and nitrogen storage, requiring the accompanying deposition of potassium. This would explain in the present studies the differences in effect on potassium balances between testosterone and estradiol.

Effects on urinary 17-ketosteroid excretion

The increases in urinary 17-ketosteroids on testosterone propionate in the present studies were in accord with previous experience (44). Reports of the effects of estrogens on 17-ketosteroids are apparently meagre. Hamblen, Pattee, and Cuyler (45) in 22 women with various types of ovarian insufficiency noted elevated "androgenic titers" and with estradiol lowered them to normal, the reductions ranging from 14 to 30 per cent. Salter, Hum, and Oesterling (46) in a female pseudohermaphrodite brought about a marked decrease (approximately 80 per cent) with 10 mgm. daily of stilbestrol. In our two male patients given estradiol benzoate the 17-ketosteroids were lowered by 22 per cent and 34 per cent, in the general range of reduction noted by Hamblen, Pattee, and Cuyler. Presumably the decreased excretion results from partial suppression of anterior pituitary production of gonadotrophin or ACTH or both.

Effects on urinary citric acid excretion

Opposing effects of androgen and estrogen were seen consistently on urinary citric acid excretion, androgen decreasing and estrogen increasing. When the steroids were combined, the usual result was a neutralization so that citric acid varied little from the control level. When the combined

steroids were stopped, frequently a rise in urinary citric acid appeared, suggesting a prolongation of the estrogen effect on this constituent.

The action of estrogen to increase urinary citric acid is an important one for the problem of urinary tract stone formation, particularly where large amounts of calcium are being excreted. Abundant amounts of citric acid in the urine are useful for assistance in keeping calcium in solution (19, 47). Thus, although testosterone alone supplies many of the principal, desired metabolic effects which would assist in the management of the clinical problem of paralysis, the addition of estrogen serves a particularly useful purpose in counteracting the lowering effect of androgen on urinary citric acid.

Testosterone was also shown to cause a small but consistent increase of 0.2 unit in urinary pH. This change, although extremely slight, is in the direction tending to be unfavorable for calcium solubility. This effect was not consistently seen on combined steroid and not at all on estradiol benzoate.

Basal metabolic rate

The somewhat limited number of tests of basal metabolic rate suggested a gradual decline in basal metabolism over the course of the first two to six months after onset; this was noted in five of the seven patients studied from within the first two months of onset. (The possibility that respiratory muscle involvement might impair the accuracy of the determinations was a factor in only one patient; the decline in his rate was similar to that of the other patients.) This downward trend in basal metabolic rate extended for approximately the duration of negative nitrogen balance and of decline in urinary creatinine; the possibility is suggested that the fall in basal metabolism occurred because of the considerable decrease in active protoplasmic mass in these patients.

More uniformly, administration of the gonadal steroids brought about an increase of 8 to 15 per cent in basal metabolism. This rise was of the same general range as the increase noted in most of the series reported to date (4, 24, 48). The few series in which an increase was not noted upon administration of this steroid were apparently those in which it was given for less than 10 days or in doses of less than 25 mgm. daily.

Clinical or side effects

In addition to their metabolic effects, gonadal hormones have various clinical effects (49–51) related mainly to their responsibility in natural production for the maleness or femaleness of the individual. Although certain undesirable side-effects may occur when a hormone is given to a patient of the same gender, by far the more disturbing effects are related to the production of symptoms or signs generally attributed to the opposite sex. These sex-related effects have been responsible for reluctance on the part of many physicians to use these hormones widely. It has not been clear whether some of these side-effects might be overcome by use of combinations of male and female hormones or only by restriction of daily or total dosage to certain limits, the latter course possibly leading at least in part to loss of useful action.

Review of the clinical or side effects from the administration of gonadal steroids in this series revealed mainly considerable variation from patient to patient in the accumulated dosage level at which these effects became evident. On testosterone, for example, two male patients developed acne after 400 and 600 mgm., respectively, and two others no noticeable effects after 1050 and 1350 mgm. The small experience in this predominantly male group of patients tended to support the idea that a 20:1 weight ratio of testosterone propionate to estradiol benzoate is neutralizing with respect to clinical effects (52); a 15:1 ratio was usually slightly feminizing and 30:1 in the one instance given was masculinizing. This tentative conclusion should not be projected to effects to be anticipated either on longer administration of steroids or in predominantly female groups of patients. In the few instances in which it was possible to observe the situation, a dosage of a hormone, which produced side-effects when given alone, did not if given again with the hormone of the opposite sex, the latter being in preponderant dosage; in these second circumstances the now preponderant hormone exerted its own clinical effects.

Comparison of effects of testosterone propionate alone and combined with estradiol benzoate

An evaluation of whether estrogen in combination with androgen exerted greater effects than androgen alone was possible in three patients and

to a limited extent in a fourth. Most observations indicated no clear advantage of combined steroid administration. Nitrogen storage was slightly greater on androgen alone in two patients and slightly more on combined steroids in one, but the significant difference with respect to nitrogen was that the rebound loss after discontinuation of hormone was considerably greater with combined steroid administration; after testosterone alone the rebound loss of nitrogen averaged only 8 per cent of the total stored on the steroid. Creatinuria was increased more by combined steroid in two patients and by testosterone alone in one; in the latter patient, glycocyamine excretion was measured under both forms of hormone administration and was found to be more increased by combined steroid. A lack of consistent pattern was also present with respect to the minor changes in urinary creatinine. Decreases in serum phosphorus and increases in urinary 17-ketosteroids were virtually the same on either form of steroid.

In calcium and citric acid metabolism, however, advantages were indicated for giving estrogen and androgen together over androgen alone, although not in every case. Allowing for the fact that more marked changes were possible during the early months when excretion of calcium was at high level, the *urinary calcium* of two patients, D.A. and R.W., was more greatly lowered (and over a shorter period) by combined steroid than by testosterone alone, particularly percentagewise. In F.S., the percentage reduction was equal by the two forms although the decrease was quantitatively greater by testosterone, given earlier than the combined. In contrast to these effects, estradiol benzoate given alone caused an increase in urinary calcium in two of the three instances in which it was given. Negative *calcium balance* was considerably more greatly reduced by combined steroid than by t.p. in one patient, R.W. In D.A., the reduction of negative balance was approximately the same by the two forms, although it may be pointed out that testosterone alone was given for six days longer. The data of F.S. were preliminarily reported (53) to show a greater reduction by combined steroid to the extent of 85 mgm. per day; this figure is correct if one compares only the control balance with the last metabolic balance period on steroid. If one takes into account, however, the frequent continuation of effect after hormone is stopped, which in this patient led to a continuing reduction in calcium excretion over the next 12 days after testosterone was discontinued, then the change in calcium balance in this patient was approximately equal by both forms of steroids. The studies of R.V.K. are difficult to compare with respect to calcium metabolism because of their short duration and the preponderant total dosage of estradiol when combined steroid was given. In the studies of J.C. in which combined steroid was given preceded by 18 days on testosterone alone, no obvious effect was noted in nitrogen, phosphorus or calcium metabolism from the addition of the estrogen to testosterone.

This lack of uniform advantage of combined steroid over testosterone alone was pointed out in a recent review (53) of twelve cases in the literature in which the effects of the two forms of steroid administration on calcium balance could be compared. In four of the twelve cases no difference was detectable, while in eight an additive effect of estrogen was present, usually equal to approximately 100 mgm. of calcium per day. Despite this lack of uniformity, the number of instances of added benefit to calcium storage (or reduced loss) from combined steroids was in the majority. Consideration should also be given to the added advantage of combined steroids for providing levels of urinary citric acid more protective of calcium solubility than those produced by testosterone alone. For these reasons, in clinical situations in which urinary tract stone formation and large losses of calcium from the skeleton are serious threats, it seems preferable to advise combined administration of testosterone and estrogen.

Application to treatment of disease

These studies were originally inititated for the purpose of increasing knowledge of the natural processes of paralytic acute anterior poliomyelitis and of possibly indicating improved methods of management. Fortunately, the evident promise of poliomyelitis vaccine makes these findings less widely applicable to infantile paralysis. Nevertheless, a number of features which have been described in these papers are presumably common, in varying degree, to other conditions, such as paraplegia or extensive injury, and the hormonal effects observed should have useful application to these disorders.

SUMMARY

The fourth paper in this series on metabolic studies in paralytic acute anterior poliomyelitis described studies of the effects of administration of gonadal steroid hormones in seven patients. During various phases of the disease, testosterone propionate was given on eight occasions for periods ranging from 12 to 42 days. Estradiol benzoate was twice given alone during the convalescent phase, for 18 and 30 days each. The two steroids were given together in six studies for periods of 12 to 36 days. The principal findings were as follows:

1. During the convalescent and chronic phases testosterone propionate alone and combined with estradiol benzoate in nine studies brought about a mean daily storage of nitrogen of 2.5 gm. From various considerations, it was thought probable that the stored protein was being deposited principally in uninvolved muscle fibers. Rebound loss of nitrogen following testosterone alone averaged 8 per cent of the total stored during steroid administration; rebound loss of nitrogen following combined steroids averaged 38 per cent. In two studies during the early convalescent phase when nitrogen excretion was high, testosterone did not bring about a clear nitrogen-sparing effect. Estradiol benzoate alone brought about an *increase* in urinary nitrogen resulting in an average daily nitrogen loss of 0.78 gm.

2. The pattern of creatinuria and increase in urinary glycocyamine on testosterone propionate, given during the convalescent and chronic phases, indicated that this steroid caused an increase in the synthesis of creatine as well as in its storage. During the early convalescent phase when creatinuria was particularly high, no increase in urinary glycocyamine occurred on testosterone propionate. The pattern of creatinuria and increase in urinary glycocyamine on estradiol benzoate suggested that this steroid caused an increase in synthesis of creatine but probably not in its storage.

3. Urinary calcium excretion and negative calcium balance were reduced by 0.100 to more than 0.300 gm. per day by both testosterone alone and combined testosterone and estradiol. Among the three patients in whom effects of the two forms of steroid on calcium balance could be compared, combined steroids were demonstrated to have a clear advantage over testosterone alone in one. Estradiol benzoate alone, in two of the three instances in which it was given, caused a slight but definite rise in urinary calcium.

4. Urinary citric acid excretion was reduced by testosterone propionate in all but one patient and increased by estradiol benzoate. When the two steroids were given together, urinary citric acid varied little from control levels. The significance of these effects for urinary calcium solubility was pointed out.

5. Serum phosphorus was reduced slightly by testosterone and by combined steroids and unchanged by estradiol; no other significant serum chemistry changes were noted.

6. Sodium tended to be stored transiently under the influence of testosterone, but mean sodium balances over the entire course of testosterone administration were virtually identical with control sodium balances; sodium diuresis occurred, however, when testosterone was discontinued. On combined steroids definite and continuing sodium retention occurred. On estradiol, sodium retention occurred in one patient and not in another. Urinary chloride excretion diminished slightly during all three forms of hormone administration.

7. Water retention averaging slightly more than 100 ml. per day occurred on testosterone alone and on combined steroids; in five studies, water balances during the first 12 days following discontinuation of these steroids were more than 300 ml. per day less positive than during steroid administration. Water retention did not occur on estradiol benzoate alone in the two males to whom this steroid was given.

8. Potassium was retained on testosterone alone and on combined steroids. Potassium balances became less positive on estradiol benzoate.

9. Observations with respect to physiological functions were as follows: Increase in basal metabolic rate on all three forms of steroid administration was 8 to 15 per cent. Gradual improvement in muscle strength during the convalescent phase did not appear to be influenced by hormone administration. Small increases in calf and thigh girth occurred on testosterone and combined steroids. Combined steroids brought about a rise in skin temperature of the feet and legs in the one patient in which this study was made; testosterone

alone caused no rise in skin temperature in the two patients so observed.

10. Considerable variation was noted in the level of accumulated dosage at which clinical masculinizing or feminizing effects appeared.

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APPENDIX A

Tables of Metabolic Balance Data

Tables I–IX.	Body Weight, Average Daily Urinary Creatinine, Creatine and Sulphur, and Nitrogen Balance (Patients D.Q., M.O'M., D.A., J.C., R.W., F.S., C.R., J.W. and R.V.K.)*
Tables X–XVIII.	Average Daily Urinary Citric Acid, and Calcium and Phosphorus Balances (Patients D.Q., M.O'M., D.A., J.C., R.W., F.S., C.R., J.W. and R.V.K.)*
Table XIX.	Lovett Method Examinations of Representative Muscle Groups Initially and at Six Months after Onset of Illness in Nine Patients with Paralytic Acute Anterior Poliomyelitis

* All excretion and balance data given in gm. per 24 hours.

TABLE I

Patient D. Q.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Days after onset	Body weight*	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	3–5	(61.0)†	1.45	0.168	0.60	3.4	11.42			− 9.0‡	Paralysis 3rd day after onset
	7, 8		1.30	0.098	0.82	6.9	16.17			−10.3	
	9–11		1.34	0.111	0.71	6.4	17.43			−12.0	
	12–14		1.42	0.168	0.78	8.7	16.76			− 9.1	Transferred to Metabolism Ward, day 14
	15–17	55.45	1.44	0.213	0.86	10.24	19.13			− 9.9	
	18–20		1.22	0.299	1.09	11.95	17.36			− 6.4	
	21–23	53.92	1.28	0.265	1.29	12.66	18.38			− 6.7	
	24–26	54.47	1.20	0.305	0.90	7.79	15.37			− 8.6	
	27–29		1.24	0.340	0.84	7.99	14.03			− 7.0	
	30–32	51.80	1.18	0.337	0.86	11.78	14.60			− 3.8‡	
2	33–44	52.30	1.13	0.255	1.00	12.81	15.35	0.90	16.25	− 3.44	Began 2,000 calories
2	45–56	51.32	1.00	0.234	0.91	12.81	13.64	1.11	14.75	− 1.94	
2	57–68	50.71	1.02	0.248	0.82	12.80	11.81	1.31	13.12	− 0.32	Began 2,400 calories
3	69–86	51.94	1.05	0.219	0.76	12.81	10.65	1.38	12.03	+ 0.78	Began sitting
3	87–104	52.58	1.08	0.148	0.67	12.81	9.05	1.39	10.44	+ 2.37	
4	105–128	53.69	1.08	0.148	0.69	12.80	9.55	1.38	10.93	+ 1.87	Began walking with crutches
4	129–152	54.88	1.17	0.131	0.76	12.80	10.42	1.40	11.82	+ 0.98	
4	153–176	55.28	1.16	0.168	0.79	12.80	10.86	1.54	12.40	+ 0.40	Walking with cane only
	177	54.65									
2	285–296	55.66	1.34	0.084	0.68	12.80	9.69	1.16	10.85	+ 1.95	
	297	55.85									

* Body weights at beginning of each set of days or metabolic periods.
† Body weight prior to onset of illness.
‡ N balances days 3 to 32 based on assumed daily fecal N value of 1.00 gm., the average analyzed fecal N loss during periods I through IV.

TABLE II

Patient M. O'M.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Days after onset	Body weight*	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	4, 5		1.10	0.150		8.4†	5.3			+2.1‡	Paralysis 3rd day after onset
	6, 7	57.06	1.01	0.150	0.39	8.4	9.26			−1.9	
	8, 10		0.95	0.660	0.69	8.4	13.22			−5.8	Oscil. bed begun, day 8
	11–13		1.00	1.012	0.92	8.4	15.67			−8.3	Transferred to Metabolism Ward, day 13
	14–16		0.92	0.923	0.76	5.67	13.11			−8.5	
	17–19	55.60	0.82	0.733	0.68	7.56	11.26			−4.7	
	20–22	52.82	0.84	0.563	0.66	9.20	11.12			−2.9	
	23–25	52.12	0.82	0.622	0.73	10.35	12.77			−3.4	
	26–28		0.86	0.577	0.80	11.27	12.60			−2.4‡	
2	29–40	51.59	0.78	0.591	0.83	11.30	12.02	1.06	13.08	−1.78	Began 1,800 calories
2	41–52	51.58	0.74	0.595	0.78	11.25	11.59	0.97	12.56	−1.31	Oscil. bed ended, day 40
3	53–70	51.67	0.68	0.577	0.76	11.23	11.43	0.90	12.33	−1.10	
3	71–88	49.96	0.65	0.523	0.70	11.23	10.68	0.82	11.50	−0.27	
2	89–100	49.80	0.64	0.323	0.62	10.80	9.26	1.00	10.26	+0.54	Began sitting
4	101–124	50.48	0.66	0.420	0.61	11.26	9.26	0.95	10.21	+1.05	Began walking, "walker"
4	125–148	50.77	0.68	0.381	0.65	11.30	9.51	0.94	10.45	+0.85	Began walking, crutches
4	149–172	50.48	0.69	0.405	0.66	11.30	9.94	0.90	10.84	+0.46	Began walking, 1 crutch
	173	50.34									

* Body weights at beginning of each set of days or metabolic periods.
† Approximate dietary intake days 4 to 13, not constant.
‡ N balances days 4 to 28 based on assumed daily fecal N value of 1.02 gm., the average analyzed fecal N loss during periods I through IV.

TABLE III

Patient D. A.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Days after onset	Body weight*	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	6, 7	(89.2)†	1.98	0.110	1.04		18.80				Paralysis 4th day after onset
	8–10		1.93	0.190	1.26	10.7	21.83			−13.0‡	
	12, 13		1.89	0.829	1.31	11.0	24.45			−15.2	Transferred to Metabolism
	14–16		1.75	0.907	1.48	11.5	24.33			−14.6	Ward, day 16
	17, 18	81.25	1.76	1.580	1.64	14.40	27.75			−15.2‡	Began 2,800 calories
(4-day)	19–22	79.76	1.46	1.270	1.38	14.37	22.96	1.83	24.79	−10.42	
(4-day)	23–26		1.27	0.997	1.40	14.37	20.90	1.83	22.73	− 8.35	
3	27–44	79.25	1.26	0.890	1.08	14.36	17.90	1.77	19.67	− 5.31	Test. prop. 25 mgm. o.d.
2	45–56	78.64	1.00	0.958	0.95	14.36	14.64	1.78	16.42	− 2.06	Test. prop. 50 mgm. o.d.
2	57–68	78.60	0.98	1.120	1.00	14.37	17.04	1.73	18.77	− 4.40	
2	69–80	76.68	0.88	0.850	0.96	14.37	14.84	1.62	16.46	− 2.09	
3	81–98	77.03	0.90	0.686	0.88	14.34	13.25	1.56	14.81	− 0.47	Began sitting
3	99–116	77.37	0.87	0.573	0.66	14.38	10.32	1.63	11.95	+ 2.43	T.p. 25 mgm.; estr.b. 1.66 mgm.
1	117–122	80.43	0.86	0.713	0.64	14.37	10.21	1.75	11.96	+ 2.41	T.p. 25 mgm.; estr.b. 3.32 mgm.
2	123–134	80.74	0.98	1.016	0.85	14.37	13.80	1.60	15.40	− 1.03	
2	135–146	78.95	0.92	0.738	0.92	14.37	13.72	1.57	15.29	− 0.92	
3	147–164	78.66	0.91	0.587	0.87	14.45	13.98	1.25	15.23	− 0.78	Began 2,000 calories
3	165–182	77.59	0.83	0.585	0.85	14.38	13.17	1.18	14.35	+ 0.03	Began standing
3	183–200	75.72	0.91	0.633	0.81	14.42	12.87	1.29	14.16	+ 0.26	Began walking, crutches
3	201–218	76.05	0.92	0.666	0.80	14.40	12.85	1.19	14.04	+ 0.36	
3	219–236	75.16	0.95	0.811	0.82	14.43	13.04	1.18	14.22	+ 0.21	Estr.b. 1.66 mgm. o.d.
2	237–248	75.75	0.90	1.008	0.86	14.42	13.93	1.24	15.17	− 0.75	Estr.b. 3.32 mgm. o.d.
2	249–260	75.18	0.88	0.866	0.88	14.41	14.00	1.32	15.32	− 0.91	
	261	72.12									

* Body weights at beginning of each set of days or metabolic periods.
† Body weight day before onset of illness.
‡ N balances days 8 to 18 based on assumed daily fecal N value of 1.80 gm., the average analyzed fecal N loss during periods I through IV.

TABLE IV

Patient J. C.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Days after onset	Body weight*	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	6, 7	82.00	1.71	0.186	0.98		16.35				Paralysis 2nd day after onset
	8, 9		1.80	0.286	1.45		22.65				
	11–13		1.69	0.452	1.54		22.52				
	14–16	78.60	1.75	1.085	1.43	14.3	24.58			−11.9†	Oscil. bed begun, day 14
	17–19		1.76	1.430	1.49	14.6	25.24			−12.3	Transferred to Metabolism
	20–22	74.75	1.77	0.919	1.07	10.9	19.63			−10.3	Ward, day 22
	23–25	73.26	1.70	1.064	1.26	12.90	19.73			− 8.4	Began 2,400 calories
	26, 27		1.69	0.850	1.04	12.80	16.86			− 5.7†	
2	28–39	73.21	1.52	0.779	1.06	12.77	16.39	1.68	18.07	− 5.30	Oscil. bed ended, day 39
3	40–57	71.68	1.35	0.718	0.99	12.77	15.48	1.57	17.05	− 4.28	
2	58–69	68.85	1.22	0.655	0.85	12.80	13.60	1.68	15.28	− 2.48	
3	70–87	68.20	1.11	0.390	0.66	12.80	9.88	1.73	11.61	+ 1.19	T.p. 25 mgm. o.d.
3	88–105	68.88	1.12	0.524	0.58	12.77	9.04	1.75	10.79	+ 1.98	T.p. 25 mgm.; estr.b. 1.66 mgm.
3	106–123	69.18	1.08	0.766	0.84	12.78	13.03	1.68	14.71	− 1.93	
1	124–129	66.72	1.05	0.378	0.65	12.80	10.71	2.18	12.89	− 0.09	Began 2,800 calories
3	130–147	66.42	1.08	0.300	0.62	12.80	9.91	1.97	11.88	+ 0.92	Began sitting
4	148–171	66.84	1.09	0.399	0.62	12.80	9.91	2.11	12.02	+ 0.78	
3	172–189	67.03	1.11	0.384	0.63	12.80	10.35	2.02	12.37	+ 0.43	Began standing
3	190–207	67.33	1.11	0.329	0.55	12.80	8.68	2.12	10.80	+ 2.00	T.p. 25 mgm. o.d.
3	208–225	69.35	1.14	0.455	0.54	12.80	8.59	2.04	10.63	+ 2.17	T.p. 25 mgm.; estr.b. 1.66 mgm.
3	226–243	70.29	1.11	0.684	0.75	12.80	12.03	1.88	13.91	− 1.11	Began walking, day 220
3	244–261	67.53	1.11	0.395	0.71	12.80	10.95	1.82	12.77	+ 0.03	
	262	67.76									

* Body weights at beginning of each set of days or metabolic periods.
† N balances days 14 to 27 based on assumed daily fecal N value of 1.61 gm., the average analyzed fecal N loss of periods I through IV.

TABLE V

Patient R. W.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance *

No. 6-day periods	Days after onset	Body weight†	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	19–21	60.32	1.35	1.316	1.30	12.72	22.36			−10.8‡	
	22–24	60.00	1.26	0.690	1.21	12.72	20.57	1.15	21.72	− 9.00	Began 2,000 calories, day 23
	25–27		1.14	0.725	1.19	12.72	18.45	1.15	19.60	− 6.88	
3	28–45	58.55	1.07	0.780	0.95	12.80	15.88	1.23	17.11	− 4.31	Test. prop. 25 mgm. o.d.
2	46–57	57.45	0.96	0.732	0.82	12.80	13.60	1.14	14.74	− 1.94	Test. prop. 25 mgm. o.d.
2	58–69	56.40	0.93	0.750	0.98	12.80	15.70	0.96	16.66	− 3.86	
2	70–81	54.59	0.93	0.707	0.89	12.78	14.68	1.13	15.81	− 3.03	
2	82–93	53.51	0.83	0.521	0.81	12.80	13.03	1.13	14.16	− 1.36	Began sitting
2	94–105	52.84	0.78	0.490	0.84	12.80	12.26	1.02	13.28	− 0.48	Began 2,400 calories
3	106–123	53.49	0.85	0.590	0.64	12.81	9.65	1.09	10.74	+ 2.07	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	124–135	55.40	0.85	0.823	0.85	12.81	12.96	1.15	14.11	− 1.30	
3	136–153	53.76	0.80	0.620	0.84	12.80	12.48	1.21	13.69	− 0.89	
3	154–171	53.36	0.77	0.522	0.80	12.78	11.79	1.13	12.92	− 0.14	Began walking
1	172–177	54.50	0.86	0.438	0.67	12.80	10.74	1.37	12.11	+ 0.69	Began 2,800 calories
2	178–189	54.82	0.84	0.464	0.72	12.79	10.65	1.19	11.84	+ 0.95	
3	190–207	55.28	0.84	0.615	0.70	12.54	10.93	1.54	12.47	+ 0.07	Estr.b. 1.66 mgm. o.d.
2	208–219	57.21	0.85	0.564	0.78	12.80	11.93	1.31	13.24	− 0.44	
2	220–231	56.29	0.84	0.302	0.72	12.80	11.08	1.16	12.24	+ 0.56	
2	232–243	57.44	0.86	0.256	0.60	12.79	8.92	1.36	10.28	+ 2.51	Test. prop. 25 mgm. o.d.
2	244–255	59.33	0.94	0.401	0.52	12.79	8.32	1.37	9.69	+ 3.10	Test. prop. 25 mgm. o.d.
2	256–267	61.05	0.96	0.574	0.68	12.80	10.96	1.48	12.44	+ 0.36	
2	268–279	59.80	0.92	0.442	0.73	12.78	10.69	1.55	12.24	+ 0.54	
	280	60.43									

* Paralysis 1st day after onset; admitted directly to Metabolism Ward.
† Body weights at beginning of each set of days or metabolic periods.
‡ N balance days 19 to 21 based on assumed daily fecal N value of 1.15 gm., the average analyzed fecal N loss during periods I through IV.

TABLE VI

Patient F. S.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance *

No. 6-day periods	Days after onset	Body weight†	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	31–33	68.91	1.20	0.980		14.55	21.02			−8.1‡	Began 2,800 calories
(7-day)	34–40	69.47	1.19	0.924	1.07	14.40	19.82	1.54	21.36	−6.96	
2	41–52	68.47	1.04	0.742	1.05	14.36	17.90	1.54	19.44	−5.08	
3	53–70	68.56	0.87	0.826	0.93	14.40	15.54	1.72	17.26	−2.83	Oscillating bed
2	71–82	70.02	0.72	0.754	0.93	14.36	14.18	1.60	15.78	−1.42	Oscillating bed
2	83–94	69.37	0.75	0.888	0.91	14.43	15.10	1.71	16.81	−2.38	
2	95–106	70.14	0.68	0.725	0.82	14.40	13.71	1.69	15.40	−1.00	
3	107–124	69.13	0.59	0.706	0.84	14.38	13.16	1.54	14.70	−0.32	Oscillating bed
3	125–142	70.46	0.59	0.760	0.83	14.39	13.22	1.60	14.82	−0.43	
2	143–154	70.43	0.59	0.749	0.84	14.38	12.50	1.71	14.21	+0.17	
3	155–172	70.57	0.66	0.764	0.69	14.39	10.98	1.46	12.44	+1.95	Test. prop. 25 mgm. o.d.
2	173–184	73.61	0.63	0.796	0.70	14.38	11.14	1.44	12.58	+1.80	Test. prop. 25 mgm. o.d.
2	185–196	74.36	0.64	0.938	0.70	14.38	10.98	1.64	12.62	+1.76	Test. prop. 50 mgm. o.d.
3	197–214	74.92	0.62	0.872	0.81	14.39	13.35	1.37	14.72	−0.33	
3	215–232	74.50	0.64	0.780	0.80	14.37	12.73	1.58	14.31	+0.06	
2	233–244	73.50	0.65	0.792	0.66	14.40	10.66	1.45	12.11	+2.29	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	245–256	76.87	0.69	0.952	0.63	14.38	9.99	1.62	11.61	+2.77	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	257–268	77.85	0.63	0.928	0.72	14.38	10.54	1.42	11.96	+2.42	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	269–280	79.00	0.71	0.886	0.88	14.40	13.36	1.57	14.93	−0.53	
2	281–292	77.85	0.66	0.779	0.92	14.37	14.14	1.78	15.92	−1.55	
	293	76.80									

* Paralysis 3rd day after onset; admitted directly to Metabolism Ward.
† Body weights at beginning of each set of days or metabolic periods.
‡ N balance days 31 to 33 based on assumed daily fecal N value of 1.63 gm., the average analyzed fecal N loss during periods I through IV.

TABLE VII

Patient C. R.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance *

No. 6-day periods	Days after onset	Body weight†	Urine			Intake N	Output N			N balance	Remarks
			Creatinine	Creatine	S		Urine	Feces	Total		
		Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
	50–55	47.50	0.69	0.884	0.76	12.80	15.20			−3.6‡	Began 2,000 calories
2	56–67	46.71	0.62	0.800	0.80	12.88	15.22	1.17	16.39	−3.51	
2	68–79	46.45	0.51	0.663	0.79	12.78	13.21	1.14	14.35	−1.57	
3	80–97	46.31	0.48	0.522	0.71	12.76	12.21	1.29	13.50	−0.74	Oscil., slow and rapid
2	98–109	46.18	0.46	0.551	0.70	12.87	11.94	1.17	13.11	−0.24	Oscil., slow and rapid
3	110–127	46.76	0.45	0.566	0.72	12.80	11.89	1.19	13.08	−0.28	
3	128–145	45.88	0.45	0.654	0.71	12.86	12.31	1.10	13.41	−0.55	Rapid rocking begun
2	146–157	45.29	0.47	0.593	0.73	12.74	11.92	1.04	12.96	−0.22	
3	158–175	45.77	0.50	0.646	0.56	12.75	9.76	1.12	10.88	+1.87	Test. prop. 25 mgm. o.d.
2	176–187	48.34	0.53	0.762	0.55	12.74	9.45	1.12	10.57	+2.17	Test. prop. 25 mgm. o.d.
2	188–199	49.19	0.51	0.770	0.54	12.76	9.28	1.07	10.35	+2.41	Test. prop. 50 mgm. o.d.
1	200–205	50.29	0.54	0.905	0.52	12.87	9.65	1.25	10.90	+1.97	
	206–208	49.10	0.50	0.894		10.96	11.82				Aspiration pneumonitis
1	209–214	49.10	0.49	0.889	0.75	12.80	13.19	1.08	14.27	−1.47	
4	215–238	48.03	0.51	0.775	0.71	12.77	12.17	1.02	13.19	−0.42	
3	239–256	46.74	0.52	0.650	0.71	12.76	11.83	1.12	12.95	−0.19	Began sitting
3	257–274	47.22	0.47	0.675	0.74	12.80	11.73	1.02	12.75	+0.05	Rapid rocking ended, day 241
2	275–286	46.78	0.52	0.684	0.74	12.74	11.55	1.10	12.65	+0.09	Began standing, day 271
2	287–298	47.01	0.52	0.588	0.60	12.74	9.50	1.11	10.61	+2.13	Test. prop. 25 mgm. o.d.
	299	49.15									

* Paralysis 1st day after onset; admitted directly to Metabolism Ward.
† Body weights at beginning of each set of days or metabolic periods.
‡ N balance days 50 to 55 based on assumed daily fecal N value of 1.15 gm., the average analyzed fecal N loss during periods I through IV.

TABLE VIII

Patient J. W.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Body weight*	Urine			Intake N	Output N			N balance	Remarks
		Creatinine	Creatine	S		Urine	Feces	Total		
	Kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
3†	48.54	0.50	0.545	0.65	12.69	10.55	1.44	11.99	+0.70	Began 1,800 calories
3†	48.50	0.48	0.549	0.61	12.83	10.29	1.34	11.63	+1.20	
3†	48.66	0.49	0.594	0.57	12.85	10.22	1.70	11.92	+0.93	Oscillating bed
3†	48.26	0.48	0.537	0.59	12.60	10.40	1.55	11.95	+0.65	Oscillating bed
3†	47.90	0.55	0.494	0.57	12.31	9.97	1.58	11.55	+0.76	
2	47.85	0.55	0.534	0.43	12.85	7.86	1.80	9.66	+3.19	Test. prop. 50 mgm. o.d.
3	48.63	0.56	0.686	0.55	12.85	9.96	1.55	11.55	+1.34	
3	48.31	0.55	0.533	0.57	12.85	10.51	1.57	12.08	+0.77	Standing with braces
	48.24									

* Body weights at beginning of each set of days or metabolic periods.
† Seven-day periods.

TABLE IX

Patient R. V. K.—Body weight, average daily urinary creatinine, creatine and sulphur, and nitrogen balance

No. 6-day periods	Body weight*	Urine			Intake N	Output N			N balance	Remarks
		Creatinine	Creatine	S		Urine	Feces	Total		
	Kg.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
3	28.30	0.21	0.611	0.43	9.35	6.72	0.97	7.69	+1.66	Began 1,595 calories
3	29.89	0.22	0.577	0.45	9.36	7.14	1.05	8.19	+1.17	
4	30.62	0.21	0.601	0.46	9.35	7.23	1.05	8.28	+1.07	Oscillating bed
3	31.86	0.21	0.601	0.49	9.35	7.40	1.05	8.45	+0.90	Oscillating bed
3	31.92	0.21	0.626	0.50	9.36	7.20	1.10	8.30	+1.06	
2	32.37	0.20	0.634	0.48	8.95	7.56	1.00	8.56	+0.39	
3	32.04	0.22	0.740	0.37	9.36	5.55	1.07	6.62	+2.74	Test. prop. 50 mgm. o.d.
4	34.93	0.22	0.747	0.47	9.36	7.45	1.09	8.54	+0.82	
2	33.98	0.20	0.744	0.60	12.04	9.32	1.20	10.52	+1.52	Began 1,938 calories
2	33.64	0.20	0.662	0.59	11.87	9.24	1.10	10.34	+1.53	
2	34.42	0.24	0.778	0.56	12.10	9.49	1.06	10.55	+1.55	Estr. b. 1.66 mgm. o.d.
2	35.36	0.23	0.954	0.46	12.05	7.63	1.13	8.76	+3.29	Estr. b. 1.66 mgm.; t.p. 50 mgm.
2	37.01	0.23	0.903	0.52	11.42	8.42	1.02	9.44	+1.98	
3	36.03 36.69	0.23	0.772	0.57	11.95	9.92	0.95	10.87	+1.08	Standing with braces

* Body weights at beginning of each set of days or metabolic periods.

TABLE X

Patient D. Q.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	3–8	0.374	0.505	0.186			0.605	1.052			Paralysis 3rd day after onset
	9–14	0.568	0.940	0.216			1.005	1.200			
	15–20	0.382	1.260	0.376			1.525	1.358			Transferred to Metabolism Ward, day 14
	21–26	0.340	0.660	0.404			1.080	1.165			
	27–32	0.406	0.985	0.368			1.285	0.995			
2	33–44	0.492	0.920	0.443	0.810	−0.333	1.414	1.016	0.532	−0.134	Began 2,000 calories
2	45–56	0.438	0.920	0.447	0.823	−0.350	1.416	0.975	0.477	−0.036	
2	57–68	0.357	0.920	0.404	0.772	−0.256	1.463	0.925	0.452	+0.086	Began 2,400 calories
3	69–86	0.342	0.921	0.351	0.774	−0.204	1.459	0.898	0.479	+0.082	Began sitting
3	87–104		0.921	0.274	0.769	−0.122	1.462	0.798	0.445	+0.219	
4	105–128	0.422	0.921	0.200	0.768	−0.047	1.462	0.814	0.429	+0.219	Began walking with crutches
4	129–152	0.510	0.920	0.151	0.801	−0.032	1.462	0.829	0.464	+0.169	
4	153–176	0.414	0.921	0.124	0.818	−0.021	1.463	0.874	0.467	+0.122	Walking with cane only
2	285–296	0.542	0.921	0.228	0.600	+0.093	1.462	0.837	0.430	+0.195	

TABLE XI

Patient M. O'M.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	4–7	0.380	1.162*	0.203			1.057*	0.632			Paralysis, day 3
	8–13	0.568	1.162	0.264			1.057	0.890			Oscil. bed begun, day 8
	14–22	0.771	0.877	0.247			0.992	0.752			Transferred to Metabolism Ward, day 13
	23–28	0.892	0.930	0.337			1.314	0.836			
2	29–40	0.926	0.921	0.370	0.845	−0.294	1.354	0.791	0.584	−0.021	Began 1,800 calories
2	41–52	1.016	0.921	0.334	0.972	−0.385	1.356	0.802	0.616	−0.062	Oscil. bed ended, day 40
3	53–70	0.947	0.921	0.358	1.012	−0.449	1.356	0.819	0.687	−0.150	
3	71–88		0.921	0.352	0.826	−0.257	1.355	0.758	0.578	+0.019	
2	89–100		0.908	0.270	0.912	−0.274	1.311	0.660	0.644	+0.007	Began sitting
4	101–124		0.921	0.250	0.904	−0.233	1.356	0.703	0.617	+0.036	Began walking, "walker"
4	125–148		0.921	0.206	0.917	−0.202	1.355	0.730	0.550	+0.075	Began walking, crutches
4	149–172		0.921	0.180	0.908	−0.167	1.356	0.718	0.612	+0.026	Began walking, 1 crutch

* Approximate dietary intake days 4 to 13, not constant.

TABLE XII

Patient D. A.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	6, 7	0.662		0.270				1.178			Paralysis, day 4
	8–13	0.542	1.127	0.338			1.492	2.012			Transferred to Metabolism Ward, day 16
	14–18	0.586	1.084	0.383			1.650	2.090			
	19–26	0.364	0.920	0.392	0.802	−0.274	1.622	1.770	0.490	−0.638	Began 2,800 calories, day 17
3	27–44	0.371	0.920	0.445	0.742	−0.267	1.642	1.342	0.497	−0.197	Test. prop. 25 mgm. o.d.
2	45–56	0.308	0.920	0.364	0.793	−0.237	1.631	1.090	0.536	+0.005	Test. prop. 50 mgm. o.d.
2	57–68	0.328	0.920	0.308	0.706	−0.094	1.642	1.265	0.518	−0.141	
2	69–80	0.264	0.921	0.317	0.746	−0.142	1.625	1.118	0.517	−0.010	
3	81–98	0.478	0.920	0.298	0.716	−0.094	1.638	1.012	0.514	+0.112	Began sitting
3	99–116	0.475	0.921	0.250	0.782	−0.111	1.639	0.859	0.499	+0.281	T.p. 25 mgm.; estr.b. 1.66 mgm.
1	117–122	0.423	0.920	0.217	0.728	−0.025	1.646	0.864	0.507	+0.275	T.p. 25 mgm.; estr.b. 3.22 mgm.
2	123–134	0.680	0.920	0.184	0.668	+0.068	1.647	1.092	0.490	+0.065	
2	135–146	0.499	0.920	0.193	0.672	+0.055	1.634	0.996	0.484	+0.154	
3	147–164	0.432	0.920	0.191	0.753	−0.024	1.547	1.004	0.500	+0.043	Began 2,000 calories
3	165–182	0.427	0.920	0.191	0.716	+0.013	1.547	0.951	0.479	+0.117	Began standing
3	183–200	0.502	0.920	0.172	0.810	−0.062	1.546	0.995	0.513	+0.038	Began walking, crutches
3	201–218	0.469	0.919	0.148	0.760	+0.011	1.543	0.872	0.542	+0.129	
3	219–236	0.820	0.920	0.184	0.763	−0.027	1.547	0.952	0.520	+0.075	Estr.b. 1.66 mgm. o.d.
2	237–248	0.975	0.920	0.186	0.690	+0.044	1.546	1.048	0.478	+0.020	Estr.b. 3.32 mgm. o.d.
2	249–260	0.915	0.920	0.146	0.746	+0.028	1.545	1.062	0.534	−0.051	

TABLE XIII

Patient J. C.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	6, 7	0.381		0.214				0.997			Paralysis, day 2
	8–13	0.726		0.334				1.764			
	14–16	0.732	1.208	0.451			1.722	1.990			Oscil. bed begun, day 14
	17–22	0.738	1.072	0.564			1.483	1.616			Metabolism Ward, day 22
	23–27	0.594	0.919	0.500			1.442	1.361			Began 2,400 calories
2	28–39	0.590	0.920	0.534	0.771	−0.385	1.434	1.432	0.540	−0.538	Oscil. bed ended, day 39
3	40–57	0.591	0.919	0.512	0.826	−0.419	1.434	1.295	0.600	−0.461	
2	58–69	0.636	0.919	0.502	0.924	−0.507	1.442	1.098	0.588	−0.244	
3	70–87	0.658	0.919	0.432	0.922	−0.435	1.438	0.926	0.574	−0.062	T.p. 25 mgm. o.d.
3	88–105	0.806	0.919	0.330	0.881	−0.292	1.436	0.869	0.507	+0.060	T.p. 25 mgm.; estr.b. 1.66 mgm.
3	106–123	0.763	0.919	0.364	0.888	−0.333	1.437	1.123	0.523	−0.209	
1	124–129	0.772	0.921	0.300	0.878	−0.257	1.425	0.848	0.535	+0.042	Began 2,800 calories
3	130–147	0.815	0.921	0.288	0.811	−0.178	1.423	0.862	0.498	+0.063	Began sitting
4	148–171	0.867	0.921	0.235	0.859	−0.173	1.423	0.873	0.536	+0.014	
3	172–189	0.871	0.921	0.229	0.832	−0.140	1.422	0.885	0.490	+0.047	Began standing
3	190–207	0.717	0.920	0.168	0.836	−0.084	1.426	0.759	0.509	+0.158	T.p. 25 mgm. o.d.
3	208–225	0.738	0.921	0.130	0.801	−0.010	1.423	0.791	0.486	+0.146	T.p. 25 mgm.; estr.b. 1.66 mgm.
3	226–243	0.768	0.921	0.134	0.752	+0.035	1.423	1.009	0.462	−0.048	Began walking, day 220
3	244–261	0.632	0.921	0.160	0.722	+0.039	1.426	0.892	0.450	+0.084	

TABLE XIV

Patient R. W.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	19–21	0.407		0.566				1.540			
	22–27	0.381	0.912	0.648	0.706	−0.442	1.359	1.330	0.504	−0.475	Began 2,000 calories, day 23
3	28–45	0.241	0.920	0.581	0.828	−0.489	1.357	1.087	0.546	−0.276	Test. prop. 25 mgm. o.d.
2	46–57	0.160	0.920	0.470	0.803	−0.353	1.366	1.036	0.516	−0.186	Test. prop. 25 mgm. o.d.
2	58–69	0.268	0.919	0.454	0.712	−0.247	1.352	1.156	0.458	−0.262	
2	70–81	0.355	0.920	0.485	0.810	−0.375	1.345	1.078	0.511	−0.244	
2	82–93	0.304	0.920	0.466	0.912	−0.458	1.353	0.978	0.361	−0.186	Began sitting
2	94–105	0.400	0.920	0.429	0.856	−0.365	1.432	0.980	0.326	−0.074	Began 2,400 calories
3	106–123	0.463	0.920	0.308	0.813	−0.201	1.436	0.780	0.523	+0.133	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	124–135	0.572	0.920	0.227	0.784	−0.091	1.435	1.052	0.497	−0.114	
3	135–153	0.416	0.920	0.266	0.866	−0.212	1.433	0.952	0.530	−0.049	
3	154–171	0.473	0.920	0.274	0.834	−0.188	1.430	0.902	0.563	−0.035	Began walking
1	172–177	0.480	0.921	0.265	0.863	−0.207	1.450	0.845	0.550	+0.055	Began 2,800 calories
2	178–189	0.480	0.920	0.242	0.787	−0.109	1.454	0.887	0.514	+0.053	
3	190–207	0.841	0.902	0.184	0.748	−0.030	1.419	0.787	0.578	+0.054	Estr.b. 1.66 mgm. o.d.
2	208–219	0.702	0.921	0.192	0.885	−0.156	1.451	0.906	0.593	−0.048	
2	220–231	0.464	0.920	0.183	0.812	−0.075	1.453	0.826	0.540	+0.087	
2	232–243	0.415	0.920	0.188	0.820	−0.088	1.452	0.742	0.521	+0.189	Test. prop. 25 mgm. o.d.
2	244–255	0.390	0.920	0.184	0.748	−0.012	1.453	0.784	0.475	+0.194	Test. prop. 25 mgm. o.d.
2	256–267	0.440	0.920	0.164	0.768	−0.012	1.453	0.984	0.494	−0.025	
2	268–279		0.920	0.198	0.744	−0.022	1.454	0.902	0.506	+0.046	

TABLE XV

Patient F. S.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	31–33	0.615		0.600				1.620			Began 2,800 calories
	34–40	0.620	0.922	0.674	0.714	−0.466	1.675	1.550	0.506	−0.381	
2	41–52	0.739	0.922	0.638	0.931	−0.647	1.634	1.471	0.615	−0.452	
3	53–70	0.801	0.922	0.608	0.864	−0.550	1.659	1.398	0.555	−0.294	Oscillating bed
2	71–82	0.788	0.922	0.586	0.893	−0.557	1.635	1.362	0.510	−0.237	Oscillating bed
2	83–94	0.761	0.922	0.564	0.825	−0.467	1.664	1.355	0.492	−0.183	
2	95–106	0.824	0.922	0.544	0.906	−0.528	1.661	1.304	0.543	−0.186	
3	107–124	0.787	0.922	0.507	0.866	−0.451	1.651	1.292	0.511	−0.152	Oscillating bed
3	125–142	0.816	0.922	0.497	0.878	−0.453	1.651	1.294	0.523	−0.166	
2	143–154	0.896	0.922	0.475	0.962	−0.515	1.647	1.262	0.550	−0.165	
3	155–172	0.833	0.922	0.415	0.893	−0.386	1.651	1.124	0.482	+0.045	Test. prop. 25 mgm. o.d.
2	173–184	0.728	0.922	0.351	0.888	−0.317	1.647	1.173	0.468	+0.006	Test. prop. 25 mgm. o.d.
2	185–196	0.626	0.922	0.306	0.893	−0.277	1.642	1.142	0.515	−0.015	Test. prop. 50 mgm. o.d.
3	197–214	0.745	0.922	0.284	0.828	−0.190	1.653	1.206	0.462	−0.015	
3	215–232	0.747	0.922	0.319	0.903	−0.300	1.643	1.199	0.520	−0.076	
2	233–244	0.735	0.921	0.294	0.802	−0.175	1.659	1.049	0.448	+0.162	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	245–256	0.800	0.922	0.247	0.862	−0.187	1.647	1.090	0.455	+0.102	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	257–268	0.900	0.922	0.211	0.698	+0.013	1.647	1.052	0.400	+0.195	T.p. 25 mgm.; estr.b. 1.66 mgm.
2	269–280	0.886	0.922	0.210	0.818	−0.106	1.659	1.209	0.475	−0.025	
2	281–292	0.694	0.922	0.223	0.746	−0.047	1.634	1.188	0.410	+0.036	

TABLE XVI

Patient C. R.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Days after onset	Citric acid	Calcium				Phosphorus				Remarks
			Intake	Output		Balance	Intake	Output		Balance	
				Urine	Feces			Urine	Feces		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
	50–55	0.965		0.676				1.220			Began 2,000 calories
2	56–67	0.893	0.921	0.710	0.807	−0.596	1.332	1.278	0.427	−0.373	
2	68–79	0.878	0.921	0.670	0.743	−0.492	1.385	1.199	0.409	−0.223	
3	80–97	0.795	0.920	0.687	0.807	−0.574	1.336	1.128	0.385	−0.177	Oscil., slow and rapid
2	98–109	0.870	0.920	0.682	0.806	−0.568	1.408	1.154	0.418	−0.164	Oscil., slow and rapid
3	110–127	0.812	0.921	0.627	0.804	−0.510	1.399	1.128	0.373	−0.102	
3	128–145	0.802	0.920	0.634	0.827	−0.541	1.385	1.206	0.401	−0.222	Rapid rocking begun
2	146–157	0.857	0.920	0.637	0.819	−0.536	1.449	1.246	0.405	−0.202	
3	158–175	0.759	0.916	0.495	0.856	−0.435	1.414	1.012	0.411	−0.009	Test. prop. 25 mgm. o.d.
2	176–187	0.592	0.920	0.362	0.862	−0.304	1.420	1.017	0.402	+0.001	Test. prop. 25 mgm. o.d.
2	188–199	0.498	0.921	0.307	0.798	−0.184	1.461	1.010	0.393	+0.058	Test. prop. 50 mgm. o.d.
1	200–205	0.462	0.920	0.306	0.875	−0.261	1.359	0.971	0.475	−0.087	
	206–208	0.480	0.895	0.225			1.270	0.787			Aspiration pneumonitis
1	209–214	0.538	0.920	0.283	0.867	−0.230	1.399	1.088	0.490	−0.179	
4	215–238	0.570	0.920	0.378	0.817	−0.275	1.424	1.095	0.438	−0.109	
3	239–256	0.537	0.920	0.405	0.880	−0.365	1.432	1.089	0.443	−0.100	Began sitting
3	257–274	0.566	0.920	0.383	0.823	−0.286	1.419	1.084	0.411	−0.076	Rapid rocking ended, day 241
2	275–286	0.434	0.920	0.373	0.838	−0.291	1.420	1.049	0.425	−0.054	Began standing, day 271
2	287–298	0.374	0.920	0.316	0.728	−0.124	1.449	0.942	0.359	+0.148	Test. prop. 25 mgm. o.d.

TABLE XVII

Patient J. W.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Citric acid	Calcium				Phosphorus				Remarks
		Intake	Output		Balance	Intake	Output		Balance	
			Urine	Feces			Urine	Feces		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
3*	0.985	0.989	0.236	0.871	−0.118	1.683	0.966	0.606	+0.111	Began 1,800 calories
3*	0.950	1.005	0.219	0.771	+0.015	1.710	0.938	0.525	+0.247	
3*	1.023	1.006	0.226	0.927	−0.147	1.707	0.980	0.597	+0.130	Oscillating bed
3*	0.839	0.996	0.190	0.815	−0.009	1.677	0.966	0.561	+0.150	Oscillating bed
3*	0.888	0.965	0.205	0.853	−0.093	1.640	0.926	0.580	+0.134	
2	0.496	1.006	0.198	0.899	−0.091	1.707	0.846	0.605	+0.256	Test. prop. 50 mgm. o.d.
3	0.607	1.006	0.155	0.797	+0.054	1.708	0.995	0.512	+0.201	
3	0.718	1.006	0.177	0.804	+0.025	1.708	0.956	0.525	+0.227	Standing with braces

* Seven-day periods.

TABLE XVIII

Patient R. V. K.—Average daily urinary citric acid, and calcium and phosphorus balances

No. 6-day periods	Citric acid	Calcium				Phosphorus				Remarks
		Intake	Output		Balance	Intake	Output		Balance	
			Urine	Feces			Urine	Feces		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
3	0.555	0.888	0.205	0.634	+0.049	1.332	0.576	0.474	+0.282	Began 1,595 calories
3	0.592	0.888	0.183	0.651	+0.054	1.330	0.611	0.476	+0.243	
4	0.602	0.888	0.142	0.669	+0.077	1.330	0.625	0.475	+0.230	Oscillating bed
3	0.687	0.888	0.126	0.582	+0.180	1.330	0.621	0.463	+0.246	Oscillating bed
3	0.646	0.888	0.143	0.609	+0.136	1.330	0.629	0.464	+0.237	
2	0.776	0.836	0.131	0.558	+0.147	1.272	0.615	0.442	+0.215	
3	0.499	0.888	0.130	0.519	+0.239	1.332	0.529	0.415	+0.388	Test. prop. 50 mgm. o.d.
4	0.525	0.888	0.108	0.548	+0.232	1.330	0.666	0.430	+0.234	
2	0.604	1.462	0.160	1.176	+0.126	1.779	0.787	0.762	+0.230	Began 1,938 calories
2	0.488	1.449	0.141	1.083	+0.225	1.758	0.783	0.662	+0.313	
2	0.616	1.473	0.180	1.042	+0.251	1.786	0.856	0.676	+0.254	Estr.b. 1.66 mgm. o.d.
2	0.578	1.468	0.180	1.016	+0.272	1.780	0.712	0.626	+0.442	Estr.b. 1.66 mgm.; t.p. 50 mgm.
2	0.606	1.357	0.162	0.927	+0.268	1.668	0.865	0.547	+0.256	
3	0.613	1.454	0.163	0.903	+0.388	1.769	0.840	0.582	+0.347	Standing with braces

TABLE XIX

Lovett method examinations of representative muscle groups initially and at six months after onset in nine patients with anterior poliomyelitis *

Patient	D. Q.		M. O'M.		D. A.		J. C.		R. W.		F. S.		C. R.		J. W.	R. V. K.
Muscle function	Initial	At 6 mos.	Initial	At 6 mos.	Initial	At 6 mos.	Initial	At 6 mos.	Initial	At 6 mos.	Initial	At 6 mos.	Initial	At 6 mos.	Initial†	Initial‡
	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R
Chest—Intercostals	5 5	5 5	1 1	3 3	1 1	3 3	5 5	5 5	4 4	5 5	3 3	4 4	S S	4 4	5 5	0 0
Trunk—Flexion	2 2	3 3	1 1	2 2	1 1	2 2	2 2	3 2	3 3	4 4	1 1	1 1	1 1	2 2	1 1	0 0
—Extension	S S	5 5	1 1	4 4	4 4	4 4	S S	4 4	2 2	4 4	1 1	3 3	0 0	0 0	2 2	1 0
Hip—Flexion	2 2	4 3	2 2	2 4	1 1	3 2	0 1	1 2	2 3	4 4	1 0	1 1	0 0	0 0	0 1	0 0
—Extension	2 2	3 4	2 2	4 4	2 1	3 1	2 1	3 2	2 2	4 4	2 1	4 2	S S	1 1	0 0	0 1
Knee—Flexion	2 2	4 4	2 3	3 4	3 1	3 1	1 1	1 1	2 2	4 3	1 0	2 0	S S	0 0	0 1	0 0
—Extension	2 3	4 5	2 2	2 4	1 3	2 4	1 1	1 1	2 2	4 4	0 0	0 4	0 0	1 1	0 0	0 0
Ankle—Plantar flexion	2 3	4 4	2 2	3 4	3 1	4 3	1 1	1 2	3 3	4 4	3 0	4 0	0 1	1 1	0 2	0 0
—Dorsal flexion	3 1	4 1	4 4	3 5	2 0	4 0	0 0	1 1	2 2	4 4	0 0	0 0	1 0	1 1	0 0	0 0
Shoulder—Abduction	5 5	5 5	2 2	2 3	1 3	3 4	4 4	5 5	0 0	1 0	0 1	3 3	1 0	5 5	4 4	2 1
Flexion	5 5	5 5	2 2	3 3	2 3	4 4	4 5	5 5	0 0	0 0	0 1	1 3	1 0	4 3	5 5	2 1
Elbow—Flexion	5 5	5 5	3 3	4 4	2 4	4 5	5 5	5 5	0 0	2 0	0 1	1 1	4 3	4 4	5 5	4 2
—Extension	5 5	5 5	3 3	4 5	2 3	4 5	4 4	5 5	0 0	2 0	0 1	0 3	2 0	4 1	4 4	2 2
Wrist—Flexion	5 5	5 5	3 3	5 5	4 4	5 5	5 5	5 5	3 0	4 0	0 3	1 4	3 3	5 4	5 5	4 2
Fingers—Flexion	5 5	5 5	3 3	5 5	5 5	5 5	5 5	5 5	4 0	4 0	1 3	2 4	3 3	4 4	5 5	4 2
Urinary creatinine, gm./day	1.45	1.15	1.1	0.7	2.0	0.8	1.8	1.1	1.35	0.8	1.2	0.6	0.7	0.5	0.5	0.2

* *Key*—5 = Normal—Complete range of motion against gravity with full resistance.
4 = Good —Complete range of motion against gravity with some resistance.
3 = Fair —Complete range of motion against gravity.
2 = Poor —Complete range of motion with gravity eliminated.
1 = Trace —Evidence of slight contractility.
0 = Zero —No evidence of contractility.
S = Spasm.

† Seven months after onset.

‡ Seventeen months after onset.

APPENDIX B

Case Summaries of Eleven Patients with Paralytic Acute Anterior Poliomyelitis

Case Summaries

1. D.Q., 21-year-old male medical student (NYH No. 456347); onset of illness Sept. 15, 1948, with fever, headache and severe low back pain; admitted to New York Hospital (Infectious Disease Ward) Sept. 16. Physical examination revealed temperature of 38.4°C. and slight stiffness of neck. Spinal fluid contained 208 white cells per mm.^{3}, 90 per cent PMN leucocytes. An i.v. infusion of N-2-thiazolyl-phenol sulfonamide (Darvisul®) was instituted on the day of admission but was discontinued because of nausea, vomiting and retching after the patient had received 4 grams, *i.e.*, one-twelfth of recommended dose. Onset of paralysis involving the lower extremities Sept. 18, third day after onset; muscle examination at maximal weakness is given in Appendix A, Table XIX. Patient's temperature reached normal on 6th day and remained below 37.5°C. (rectal) thereafter. Paralysis of the urinary bladder required an in-dwelling catheter for 9 days; a resulting urinary infection was eradicated by sulfadiazine. Furmethide® was employed effectively on 6 occasions in doses of 1 to 3 mgm. in order to augment bladder contraction. The patient was transferred to the Metabolism Ward on Sept. 29 (14th day after onset). Marked constipation and anorexia delayed institution of fixed dietary intake until 32nd day (Table I).

Hot pack therapy for 3 hours daily was begun on the 5th day after onset and continued for two and one-half months. Muscle re-education was begun on 19th day with the patient in bed, and from 54th day this therapy was given in the pool. From 79th day physical therapy was given in the gymnasium as well as in the pool. Increase in appetite required elevation of dietary intake on 57th day from 2020 calories to 2400, the protein and calcium intake remaining unchanged.

The patient began sitting in a chair on 69th day, began standing on 78th day, walking with "walker" on 99th day, with crutches on 105th day and with the aid of only a cane on 153rd day. Muscle examination at 6 months after onset is given in Appendix A, Table XIX.

The patient was discharged on 180th day and thereafter received physical therapy as an out-patient until readmission from 282nd to 298th days for further metabolic observations. The patient returned to medical school, completed his course and is now carrying out residency duties with occasional use of cane.

2. M.O'M., 21-year-old female secretary (NYH No. 553257); onset of illness Oct. 6, 1949, with sore throat, headache, nausea, vomiting, fever, and history of left chronic otitis media; admitted to New York Hospital (Infectious Disease Ward) Oct. 7. Physical examination showed temperature of 39.5°C., perforated left ear drum draining purulent material, slight pharyngeal injection and moderate stiffness of neck. Spinal fluid contained 330 white cells per mm.^{3}, 60 per cent lymphocytes. Onset of paralysis Oct. 9, third day after onset, with weakness of left deltoid, and following day weakness of intercostals and lower extremities. V.C. on 5th day was 1250 ml. Nasal oxygen given 5th and 6th days. Temperature reached normal on 6th day and remained normal thereafter, except for minimal elevation 13th to 15th days, reason not evident. Muscle examination at maximal weakness is given in Appendix A, Table XIX. The patient was transferred to the Metabolism Ward on Oct. 19, 13th day after onset. The patient was able to take a constant dietary intake beginning on the 25th day (Table I).

Special procedures: M.O'M. was placed on a Sanders oscillating bed on the 8th day after onset. The bed oscillated 12 to 15 (average 14) hours daily through a total arc of 21 degrees (2 head down to 19 feet down). Oscillating time each day could not be extended because oscillation was too uncomfortable with patient on her side, due to tightness and pain in the shoulders; patient was able to sleep with bed oscillating when lying on back. Oscillation was discontinued on the 40th day after onset after a total period of 33 days.

Standard therapy and course: Hot pack therapy for 2 hours daily was begun on the 3rd day, was supplemented with hot tub 20 minutes daily beginning on the 6th day; packs were continued for two and one-half months. Passive motion was begun on the 5th day. Muscle re-education was started on the 14th day in bed, from 71st day was given in the pool and from 92nd day in gymnasium as well as pool.

Ventilatory function remained stationary until the 20th day, then steadily improved, V.C. on the 29th day being 1830 ml., on the 86th day 2075 ml. The patient began sitting in chair on 89th day, began standing 92nd day, walking with "walker" 101st day, with crutches 125th day, with one crutch 149th day and with one cane 167th day. Muscle examination 6 months after onset (time of discharge) is given in Appendix A, Table XIX.

The patient was discharged on 174th day and thereafter received physical therapy as an out-patient. Eleven months after onset the patient was re-employed part-time as a secretary and six months later resumed full-time secretarial work.

3. D.A., 28-year-old male manager and owner of a woodworking business (NYH No. 478821); onset of illness Sept. 16, 1948, with severe occipital headache and fever which persisted and were later accompanied by low back pain and nausea. Onset of paralysis involving all four extremities Sept. 20, fourth day after onset. Admitted to New York Hospital (Infectious Disease Ward) September 21. Physical examination revealed temperature of 39.2C., and generalized pronounced muscle weakness as shown in Appendix A, Table XIX. Spinal fluid contained 128 white cells per mm.^{3}, 96 per cent lymphocytes. Temperature reached normal on 9th day and remained normal from 11th day on. The patient was trans-

ferred to the Metabolism Ward on Oct. 2nd (16th day after onset). A constant dietary intake was begun the following day (Table I).

Special procedures: Testosterone propionate 25 mgm. i.m. daily was given beginning on the 27th day after onset. This dosage was continued for 18 days, then was raised to 25 mgm. b.i.d. for 12 days. Testosterone was discontinued on the 56th day, sesame oil 1 ml. b.i.d. i.m. being given for the next 12 days. Combined androgen and estrogen administration was begun on the 99th day with testosterone propionate 25 mgm. and Progynon-B (alpha estradiol benzoate) 1.66 mgm. (10,000 rat units), both i.m. daily. After 18 days Progynon-B dosage was doubled to 3.32 mgm. once daily, and this was given with testosterone for 6 days, both medications being discontinued on the 122nd day. After an interval of three months during which the patient made considerable improvement in muscle function and became able to walk with crutches and a long leg brace (detailed below), the patient was given Progynon-B 1.66 mgm. i.m. daily beginning on the 219th day; this dosage was continued for 18 days, then raised to 3.32 mgm. daily (1.66 mgm. twice daily) for an additional 12 days and discontinued on the 248th day. Estrogen administration was accompanied by slight nipple tenderness and the development of 3 by 3 cm. firm, flat, minimally tender tissue beneath the areolae.

Standard therapy and course: Hot pack therapy 4 to 6 hours daily was begun on the 7th day after onset and continued for two and one-half months. Muscle re-education was begun on the 18th day in bed, from the 75th day was given in the pool and from 81st day in gymnasium as well as pool. The patient also began sitting in chair on 81st day, began standing and walking in pool on 90th day. On 147th day dietary intake was reduced from 2800 calories to 2000 calories, nitrogen and calcium remaining the same, for the purpose of halting patient's perceptible increase in fat deposition. The patient began standing and walking a few steps with "walker" on 165th day, with crutches and long leg brace on 183rd day. Muscle examination at 6 months is given in Appendix A, Table XIX. At time of discharge (262nd day) patient was beginning to take few steps with crutches and without brace. At present patient walks with two canes and short drop-foot brace on right foot, works as a building contractor.

4. J.C., 39-year-old male advertising art director (NYH No. 549172); onset of illness August 17, 1949, with marked fatigue, mild abdominal and low back ache. Onset of paralysis involving chiefly the lower half of patient's body Aug. 19, second day after onset. Admitted to New York Hospital (Infectious Disease Ward) Aug. 22. Physical examination showed temperature 38.6°C., slight stiffness of neck and muscle weakness as shown in Appendix A, Table XIX. Spinal fluid showed 52 white cells per mm.^{3}, 68 per cent lymphocytes. The patient had urinary retention first 48 hours of hospital stay, requiring catheterization every 8 hours. Temperature reached normal on 8th day after onset and remained normal from 9th day on. The patient was transferred to Metabolism Ward Sept. 8 (22nd day after onset) and constant dietary intake was begun the following day (Table I).

Special procedure: J.C. was placed on a Sanders oscillating bed on the 14th day after onset. The bed was set to rock through an arc of 21 degrees (1 degree head down and 20 feet down), one and one-half minutes per cycle, and continued for a total of 16 to 18 hours (average 17) daily, being stopped only for meals, baths, bedpan, muscle re-education and briefly for application of hot packs. Oscillation was stopped on the 39th day after a total period of 26 days.

On the 70th day testosterone propionate 25 mgm. i.m. daily was begun, and this was continued for 36 days; testosterone administration was accompanied by an increase in the number of erections without nocturnal emissions and by transient acne. For 18 days during latter half of testosterone administration Progynon-B 1.66 mgm. i.m. daily was given also, accompanied by the transient appearance of tiny capillary hemangiomata over the anterior chest and upper abdomen and transient nipple soreness. Both hormones were discontinued on the 105th day, followed by a few days of fullness and tenderness of the areolae. After an interval of three months during which the patient improved to the point of beginning to stand between parallel bars (detailed below), testosterone propionate was again given, 25 mgm. i.m. daily for 36 days, beginning on the 190th day. Progynon-B, 1.66 mgm. i.m. daily, was added during the last 18 days of testosterone. Slight tenderness about the areolae and firm, flat tissue 3 by 3 cm. in area under the areolae was noted during the last 10 days of hormone administration and for approximately 10 days after both hormones were discontinued (225th day). During the last week of administration of the hormones the patient was beginning to take a few steps with long leg braces and crutches.

Standard therapy and course: Hot pack therapy 3 hours daily was begun on the day after admission, was supplemented with hot tub 20 min. daily beginning on the 20th day; prone and pin-on packs were continued for 3 months. Passive motion and muscle re-education were begun on 13th day; from the 58th day muscle re-education was given in the pool and from 133rd day in gymnasium as well as pool. Because of continuing weight loss and excellent appetite, dietary intake on 124th day was raised from 2400 to 2800 calories, the protein and calcium intake remaining unchanged. The patient began sitting in chair on 130th day, began standing and taking few steps in pool on 150th day, standing in gym 172nd day. Muscle examination 6 months after onset is given in Appendix A, Table XIX. Walking a few steps with long leg braces and "walker" was begun 214th day, and with crutches as well 220th day, being very gradually increased during the eighth month.

Patient was discharged on 262nd day and thereafter received physical therapy as an out-patient. He returned to his office half-time shortly after discharge and since 1951 has been working full-time, chiefly from wheelchair.

5. R.W., 17-year-old male high school senior (NYH No. 522078); onset of illness Oct. 1, 1948, with headache, muscle aching, chilly sensations, fever and nausea.

Onset of paralysis, involving chiefly patient's right arm, Oct. 2, first day after onset. Admitted to Grasslands Hospital, White Plains, N. Y., Oct. 2, where during next two days muscle weakness increased, involving all four extremities. Hot pack therapy 1 to 2 hours daily and passive exercise were instituted on 3rd day; by the end of first week some improvement was noted in muscles of right leg.

Patient was transferred to New York Hospital, Metabolism Ward, Oct. 19, 18th day after onset. Physical examination revealed normal vital signs and pronounced generalized muscle paralysis as shown in Appendix A, Table XIX. A constant dietary intake was begun on 23rd day (Table I).

Special procedure: Testosterone propionate 25 mgm. i.m. daily was begun on the 28th day after onset and continued for 30 days; sesame oil 1 ml. i.m. was then given during the next 6 days. During testosterone administration there was a slight increase in a mild acneform folliculitis over the anterior chest wall but no acne on face, edema or other clinical effects. On the 106th day after onset testosterone propionate 25 mgm. i.m. and Progynon-B 1.66 mgm. (10,000 rat units) i.m. daily were begun and administered for 18 days; during this administration the patient had minimal nipple soreness for the last two days but no other clinical effects. Progynon-B alone, 1.66 mgm. i.m. daily, was given for 18 days beginning on the 190th day; at this time the patient was walking well without support. On the final day of Progynon-B administration the patient noted slight tenderness of the nipples, and firm, flat tissue (2 cm. in diameter) could be palpated below each areola. Testosterone propionate alone, 25 mgm. i.m. daily, was given for 24 days beginning on the 232nd day; only symptom or sign noted was difficulty starting urinary stream upon awakening in the morning without any increased tendency to erections.

Standard therapy and course: Hot pack therapy, 4 hours daily, was continued for the first two and one-half months. Muscle re-education in bed was begun 24th day, from 87th day was given in pool and from 152nd day in gymnasium as well as in pool. Patient began sitting in chair, right arm in airplane splint, on 84th day, began standing and walking in pool on 94th day. Dietary intake was raised from 2000 to 2400 calories on 94th day, protein and calcium intake remaining unchanged. Patient began walking, without need for support, on 154th day; at this time sitting in chair was increased from 2 to 6 hours daily. By 172nd day progressive increase in activity required increase in constant dietary intake to 2800 calories, protein and calcium intake being maintained at original level.

Muscle examination at 6 months after onset (Appendix A, Table XIX), indicated progressive improvement in muscles of lower extremities and increasing facility in use of left hand, without change in right upper extremity. Patient was discharged on 281st day to continue physical therapy as an out-patient at a hospital near his home. Patient completed high school and at present is employed at office work.

6. F.S., 20-year-old male student (NYH No. 491299); onset of illness Sept. 23, 1947, with headache, stiff neck, pain in back, fever, and loose stools. Onset of paralysis, chiefly involving the legs, Sept. 26, third day after onset. Admitted to Grasslands Hospital, White Plains, N. Y., Sept. 27. Spinal fluid showed 164 white cells per $mm.^3$, 94 per cent lymphocytes. Extent of paralysis increased to involve all four extremities; paresis of urinary bladder requiring catheterization was present from 6th to 14th day. Hot pack therapy was begun shortly after admission.

Patient was transferred to New York Hospital, Metabolism Ward, Oct. 22, 29th day after onset. Physical examination revealed normal vital signs and marked, extensive muscle paralysis as shown in Appendix A, Table XIX. Constant dietary intake was begun on 31st day (Table I).

Special procedures: F.S. was placed on a Sanders oscillating bed on the 53rd day after onset. Arc of excursion of the bed was 19 degrees during first 20 days of oscillation then, by transfer to another bed, was 21 degrees (1 degree head down and 20 degrees feet down) with one complete cycle every one and one-half minutes. Oscillation was for 21 hours daily. At foot-down end of excursion of bed, blood pressure cuff under heels showed an increase in pressure of 70 mm. Hg over that in the horizontal position. Oscillation during sleep was well tolerated. Edema of ankles was noted approximately two weeks after oscillation was begun. Oscillation was discontinued after 30 days, and edema subsided within three days. The patient was again placed on an oscillating bed on the 107th day, the arc of excursion being 1.5 degrees head down to 18 degrees feet down. Slight ankle edema again occurred during oscillation and subsided three days after oscillation was stopped. This second phase of oscillation extended for 18 days.

On the 155th day testosterone propionate 25 mgm. i.m. daily was begun. This dosage was continued for 30 days; during the next 12 days 25 mgm. was given b.i.d. and then androgen was discontinued. During testosterone administration slight ankle edema occurred, and the patient noted increased beard growth, libido and more frequent erections and nocturnal emissions. Combined androgen-estrogen was given for 36 days beginning at the 233rd day; the daily dosage throughout was 25 mgm. of testosterone propionate and 1.66 mgm. of Progynon-B. Midway during the administration of these hormones the patient had slight nipple soreness for two days, but no other clinical effects were noted.

Standard therapy and course: Hot pack therapy, 6 to 8 hours daily, was continued for the first two and one-half months. Passive exercises and muscle re-education in bed were begun 35th day after onset, from 143rd day were given in pool. Improvement in muscle function was slow, as indicated in muscle examination at 6 months (Appendix A, Table XIX). Metabolic observations were discontinued after 294 days, at which time the patient was transferred to a general medical ward for continuation of physical therapy. Patient was then mobilized to chair and began standing in pool. Ultimate rehabilitation of pa-

tient has been to wheelchair from which he operates a physicians' call service.

7. C.R., 18-year-old male high school student (NYH No. 493453); onset of illness Sept. 17, 1947, with back pain, nausea, headache, stiffness of neck and fever. Onset of paralysis, initially involving left leg, Sept. 18, first day after onset. Admitted to Willard Parker Hospital, New York City, Sept. 18. Spinal fluid showed 350 white cells per $mm.^3$, "mostly mononuclears." Muscle weakness progressed to involve all four extremities, urinary bladder to the extent that catheterization was twice required on 3rd day and the respiratory muscles so that on 5th day patient was placed in tank respirator. Time out of respirator was gradually increased so that after 2 weeks he was out 30 to 45 minutes every 4 hours. Respirator was discontinued altogether on 31st day; total number of days in respirator, 26. Duration of fever was 10 days. Lay-on hot packs were given approximately 4 hours daily and muscle re-education begun during stay at Willard Parker Hospital. During 4th to 7th weeks gradual improvement in arms and hands occurred.

Patient was transferred to New York Hospital, Metabolism Ward, Nov. 4, 48th day after onset. Physical examination revealed normal vital signs except for respiratory rate of 28/min. Muscle examination reported in Appendix A, Table XIX indicates the extreme extensive muscle paralysis. Pulmonary ventilation was carried on by the action of sternocleidomastoid muscles with assistance of diaphragm, which, on fluoroscopy, moved 1 cm. bilaterally (not paradoxical); chest cage was rigid, intercostals spastic. Vital capacity 500 ml. (serum CO_2 combining power 61 vol. per cent). Urine was normal except for 10 to 20 white cells/HPF cent. and numerous bacteria; urine culture, *E. coli.* Sulfadiazine 1 gm. 4 i.d. with sodium bicarbonate 1 gm. 4 i.d. for 6 days, 62nd to 67th, cleared urinary infection. Constant dietary intake (Table I) was begun on 50th day.

Special procedures: The patient was placed on a Sanders oscillating bed on the 80th day after onset. The daily period on this bed averaged 14 hours (6 p.m. to 8:30 a.m.); arc of excursion was 20 degrees (2 degrees head down and 18 degrees feet down) with one complete cycle every one and one-half minutes. The patient slept well while the bed was oscillating. The same day, because of ventilatory insufficiency with diminished diaphragm excusion, the patient was placed on a rapid-rocking bed, rate 16/min., total arc 24 degrees (12 degrees each side of horizontal), 7 to 8 hours daily; the head of the bed was elevated so that the patient's head did not fall below the horizontal. Both forms of oscillation were discontinued after 30 days, on the 109th day. Rapid rocking only was resumed on the 128th day at the same arc and rate but for 12 hours daily; after 114 days during which time other procedures were carried out, rapid rocking was discontinued on the 241st day. Vital capacity at this time was 2400 ml.

Testosterone propionate, 25 mgm. i.m. daily, was begun on the 158th day. After 30 days the dosage was raised to 25 mgm. b.i.d. which was given for the next 12 days. Slight puffiness of ankles occurred approximately two weeks after start of therapy, but no other clinical effects were noted. Testosterone propionate, 25 mgm. i.m. daily, was again given beginning at the 287th day for 12 days; no clinical effects were noted.

Standard therapy and course: Hot pack therapy, 6 to 8 hours daily, was resumed on 51st day and, because of extreme muscle tightness, was continued for four and one-half months, to 139th day. Muscle re-education in bed was resumed on 52nd day, from 146th day was given in pool and from 239th day in gymnasium as well as pool. The slow improvement in muscle function is indicated in the muscle examination at 6 months (Appendix A, Table XIX).

On 206th day after onset, following immersion in pool, patient had malaise, nausea, temperature elevation to 39.2°C. and slight suppression of breath sounds at right base in posterior axillary line without evidence of infiltration by X-ray. Fifty thousand units aqueous penicillin q. 3 h. for 72 hours resulted in prompt clearing of signs. Duration of temperature elevation was 36 hours without evident effect upon excretion of metabolic constituents.

Patient began sitting in chair and standing in pool on 239th day. Patient stood in braces on ward on 271st day and was gradually able to shift weight from one leg to other while standing for 20 to 30 minutes daily. Patient was discharged on 301st day for two-week leave of absence, then readmitted to a general medical ward for continued physical therapy. Ultimate rehabilitation has been chiefly wheelchair.

8. J.W., 20-year-old housewife and mother (NYH No. 466391); onset of illness June 27, 1946, with backache, pains in legs, fatigue and fever. Onset of paralysis, chiefly involving legs, June 30, 3rd day after onset. Admitted to St. Mary's Hospital, West Palm Beach, Florida, where muscle weakness increased to involve abdomen, back, left shoulder and hand; for two weeks function of urinary bladder and intestinal tract was impaired so that catheterization and enemas were required. Hot pack therapy, 2 to 4 hours daily, and muscle re-education were given throughout patient's 11-week stay in St. Mary's Hospital. Patient began sitting in chair 9 weeks after onset.

Patient was transferred to New York State Reconstruction Hospital, West Haverstraw, N. Y., October 7, 102nd day after onset. Muscle re-education in pool was resumed and walking in pool with braces and walker was begun 4 weeks later.

Patient was transferred to New York Hospital, Metabolism Ward, January 19, 1947, 206th day (7 months) after onset. Physical examination revealed normal vital signs and rather marked muscle paralysis of lower extremities as indicated in muscle examination given in Appendix A, Table XIX. X-rays of the skeleton revealed osteoporosis of the pelvis and of the ends of the long bones. Constant dietary intake was begun on 212th day (Table I).

Special procedures: The patient was placed on a Sanders oscillating bed on the 258th day. Arc of excursion was 2 degrees head down to 15 degrees feet down,

cycle 2 minutes, 17 to 18 hours per day. Oscillation was discontinued after 42 days. The patient was comfortable and slept well on the bed.

Testosterone propionate 25 mgm. i.m. b.i.d. (total 50 mgm. daily) was given for 12 days beginning at the 321st day; no clinical effects were noted.

Standard therapy and course: Muscle re-education in gymnasium was begun on admission. Patient sat in chair one and one-half hours daily until 351st day when time sitting was increased and patient began standing and walking in long leg braces in "walker." Patient was discharged on 370th day to continue physical therapy as an out-patient.

9. R.V.K., 19-year-old female student nurse (NYH No. 460945); onset of illness July 9, 1945, with fever, muscular aching and weakness. Onset of paralysis involving all four limbs, July 10, on which day she was admitted to Willard Parker Hospital, New York City. Degree of paralysis increased and on 4th day after onset patient was placed in tank respirator in which she remained for 17 days. Additional treatment in Willard Parker Hospital included hot packs and hot tub 15 minutes daily. By 6 weeks after onset patient could move head from side to side and move left arm by crawling with fingers.

On August 21, 43rd day after onset, patient was transferred to New York State Reconstruction Hospital, West Haverstraw, New York, where hot packs were continued for an additional 5 weeks. Muscle re-education in bed and in pool was given daily with slow improvement in muscle function, initially in the left arm, later neck flexors, right arm and leg. In September, 1946, 14 months after onset, patient began sitting in chair 2 hours daily.

Patient was transferred to New York Hospital, Metabolism Ward, November 17, 1946, 496th day (17 months) after onset. Physical examination revealed normal vital signs, marked malnutrition (body weight 28.7 Kg.) and extensive muscle paralysis as indicated in muscle examination given in Appendix A, Table XIX. X-rays of the skeleton revealed osteoporosis of the pelvis and long bones, more pronounced at the ends of the latter. Intravenous pyelography revealed no evidence of urinary tract calculi. Since the patient at rare intervals had slight regurgitation into the nose, fluoroscopic examination of the esophagus was performed but was negative. Fluoroscopy of the chest revealed an elevated and fixed right diaphragm. Constant dietary intake (Table I) was begun on 503rd day.

Special procedures: R.V.K. was placed on a Sanders oscillating bed on the 543rd day after onset. Oscillation was continued for 42 days. The excursion was 23 degrees (head down 3 degrees, feet down 20 degrees), the rate one cycle every 150 seconds (excessive vibration at more rapid rate) and the daily period eighteen and one-half hours. During oscillation the patient slept better than previously and stated that her feet were always warm; one week after cessation of oscillation patient complained of coldness of feet.

On the 615th day testosterone propionate 25 mgm. b.i.d. (total 50 mgm. daily) was begun and was given for 18 days. Slight huskiness of the voice and a small amount of facial acne appeared 13 days after the beginning of testosterone administration.

Progynon-B, 5000 rat units, i.m. b.i.d. (total 1.66 mgm. daily) was begun on the 671st day and continued for 24 days. During the final 12 days of estrogen administration testosterone propionate, 25 mgm. i.m. b.i.d. (total 50 mgm. daily), was also given. On the final day of combined hormone administration slight huskiness of the voice was noted; this subsided in three days.

Standard therapy and course: Muscle re-education and streching in bed and in gymnasium were begun on entry. There was slight improvement in muscle function, chiefly in the left arm, during patient's 8-month stay. R.V.K. sat in chair one and one-half hours daily until last 3 weeks of her stay (719th day) when time sitting was increased gradually to 6 hours and she began standing in aluminum long leg braces 5 to 15 minutes once a day. Patient was discharged home on 736th day after onset.

10. R.Wil., 19-year-old female artist (NYH No. 552-829); onset of illness Oct. 12, 1949, with severe headache, backache, weakness, dizziness, stiff neck, and fever (T—102°F.). Onset of paralysis the following day involving both legs. Admitted to City Hospital, New York City, Oct. 13, where L.P. was performed; spinal fluid slightly cloudy, Pandy +, 200 cells. Transferred to New York Hospital Oct. 14. Patient had diabetes mellitus known for 2 years, taking 25 units of protamine zinc insulin daily. Physical examination showed muscle weakness which was severe of all leg muscles (O to P), moderately severe of trunk and mild of arm muscles (F to G). An indwelling catheter in the urinary bladder was required for four days. V.C. was 1500 ml. on Oct. 14, thereafter rose toward normal levels. The entire study was carried out on the Infectious Disease Ward where 24-hour urine collections, daily dietary survey and regulation of caloric, protein and calcium intake levels were begun on the 4th day after onset.

Special procedure: The patient was placed on a Sanders oscillating bed on the 7th day after onset. Arc of excursion was 21 degrees (1 degree head down and 20 feet down), and time for complete cycle was one and one-half minutes; daily time period oscillating varied widely but averaged 10 hours. Oscillation was discontinued on the 23rd day after a total period of 17 days.

Standard therapy and course: Pin-on hot packs, supplemented by hot tubs, were given 8 hours daily from the 4th day. Muscle re-education was started on the 16th day in bed, was given in the pool from the 37th day. The patient's diabetes was well controlled on 40 units of regular and 20 units of protamine zinc insulin daily given in the same syringe. On the 51st day urine collections were discontinued and the patient began sitting in a wheelchair. At this time shoulder girdle and arm muscles had improved generally to a rating of good; trunk and leg muscles were unchanged. Shoulder and arm muscles

continued to improve. During the 5th month after onset the patient was transferred to another hospital.

11. J.L., 21-year-old housewife (NYH No. 455795); onset of illness Oct. 1, 1948, with headache, nausea, muscle aching, stiff neck and fever (T—103°F.). Weakness of the left arm was noted on day of onset, and on the following day weakness of right arm and neck muscles with slight difficulty in breathing and swallowing. On the 5th day the patient was admitted to a local hospital where spinal fluid showed 67 cells, 90 per cent lymphocytes, and hot pack therapy was begun. On the 6th day the patient was transferred to the New York Hospital. Physical examination revealed normal vital signs except for a respiratory rate ranging from 20 to 28/min. There was extreme weakness (O to T) of all neck, shoulder, arm and intercostal muscles; trunk and abdominal muscles were rated fair and the lower extremities normal. Speech was slightly nasal and the V. C. 550 ml. Nasal oxygen was given for 5 days and rapid rocking from the 9th to the 17th day. On the 12th day 24-hour urine collections, daily dietary survey and an effort at regulating the patient's caloric, protein and calcium intake levels were begun; the entire study was carried out on the Infectious Disease Ward.

Special procedure: The patient was placed on a Sanders oscillating bed on the 24th day after onset. Arc of excursion was 18 degrees (1 degree head down and 17 feet down), and time for complete cycle one and one-half minutes; daily time period oscillating averaged 16 hours. Oscillation was discontinued on the 44th day after a total period of 21 days.

Standard therapy and course: Hot pack therapy was continued for the first two months. Passive motion and muscle re-education were begun in bed on the 17th day and from the 31st day were given in the pool. The patient began standing in the pool with water to axillae on the 35th day, began sitting in a chair 60th day, and began walking (unsupported) on the 64th day. Ventilatory capacity improved gradually and V. C. was 1000 ml. on 64th day. Patient was discharged home on the 71st day and thereafter received physical therapy as an outpatient.